Allergy, Asthma & Clinical Immunology 2006, **2**:1
http://www.aacijournal.com/content/2/4/1

ALLERGY, ASTHMA & CLINICAL IMMUNOLOGY

MEETING ABSTRACTS Open Access

Meeting program

Meeting Program

Canadian Society Of Allergy And Clinical Immunology Annual Scientific Meeting October 26 To 29, 2006 Final Program Target Audience

The Canadian Society of Allergy and Clinical Immunology (CSACI) Annual Meeting is designed for clinicians, researchers, trainees/students, and allied health professionals involved and/or interested in the study of allergy, asthma, and immunology.

Objectives

Upon completion of the Annual Meeting, participants should be able to discuss the latest advances in the research, diagnosis, and treatment of allergic and immunologic disease.

Please refer to the individual session descriptions in this program for additional, more detailed learning objectives.

Maintenance of Certification - Maintien Du Certificat

This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of The Royal College of Physicians and Surgeons of Canada.

As an accredited provider, the Canadian Society of Allergy and Clinical Immunology has approved this program.

La présente activité constitue une activité de formation collective agréée conformément è la définition précisée dans le programme de Maintien du certificat du Collège royal des médecins et chirurgiens du Canada.

En tant que prestataire agréé, la société canadienne de l'allergie et l'immunologie clinique a approuvé ce programme.

Accredited Group Learning Activity (Section 1) *Activités De Formation Collective Agréées (Section 1)*

The Royal College of Physicians and Surgeons of Canada has contributed to the sponsorship of this meeting through a National Specialty Society Annual Meeting Grant.

Le Collège royal des médecins et chirurgiens du Canada commandite la présente réunion par l'octroi d'une Subvention pour la réunion annuelle d'associations nationales de spécialistes.

Thursday, October 26, 2006

0900-1700 CSACI/ALLERGEN TRAINEE DAY

Chairs: Drs. Judah Denburg and Kent HayGlass

THIS SESSION WILL BE HELD AT THE INTER-CONTINENTAL HOTEL

360 Rue St-Antoine Ouest - *RoomSaint-Jacques* - 3rd Floor

0745-0845 **Breakfast** - *Saint-JacquesFoyer*

0845-0900 **Welcome**

Dr. Kent HayGlass, Chair, CAIDATI, University of Manitoba, Winnipeg, MB

0900-0930 **Keynote Presentation: *"Immunology Platform of the Allergen Birth Cohort"***

Dr. Mike Cyr, McMaster University, Hamilton, ON

0930-0940 **Q and A**

0940-1025 **Trainee Oral Presentations**

1040-1130 **Break and Poster Viewing**

1130-1245 **Trainee Oral Presentations**

1300-1400 **Luncheon** -*Saint-JacquesFoyer*

1400-1430 **Keynote Presentation:***"Epigenetics: What's the Buzz?"*

Dr. Ruey Su, Post-doctoral Fellow, University of Manitoba, Winnipeg, MB

1430-1440 **Q and A**

1440-1540 **Trainee Oral Presentations**

1555-1645 **Poster Viewing Reception** - *Saint-JacquesFoyer*

1645-1700 **Poster Awards and Adjournment**

ALL OTHER SESSIONS WILL BE HELD AT THE FAIRMONT THE QUEEN ELIZABETH HOTEL

1200-1300 LUNCHEON - Mackenzie

1300-1600 NON-PROFIT SUMMIT (Closed) - Mackenzie

Chair: Dr. Eric Leith

1600-2000 REGISTRATION - Mezzanine

1615-1750 CAAIF BOARD OF DIRECTORS MEETING (Closed) - Mackenzie

1800-2100 CSACI BOARD OF DIRECTORS MEETING (Closed) - Mackenzie

1830-2100 NATIONAL RESIDENT EDUCATIONAL PROGRAM (NREP) DINNER AND MEETING (Closed) -

Matapedia

Chairs: Drs. Sean Mace and Per Lidman

Friday, October 27, 2006

0700-1630 REGISTRATION - Mezzanine

0715-0830 PLENARY BREAKFAST SYMPOSIUM* - Marquette/Jolliet

Sponsored by Novartis Pharmaceuticals Canada Inc.

Topic: Severe Asthma: Pathophysiology and Management

Speaker: Dr. Robert Schellenberg, UBC Pulmonary Research Lab, Vancouver, BC

Chair: Dr. Allan Becker

0800-1600 VISIT EXHIBITS - Hochelaga 1-3

0830-0840 OPENING REMARKS BY THE PRESIDENT OF THE CSACI - Grand Salon

Dr. Susan Waserman

0840-1010 PLENARY SESSION I - FOOD ALLERGY - Grand Salon

Chairs: Drs. Susan Waserman and Zave Chad

0840-0910

Speaker: Dr. Scott Sicherer, Jaffe Food Allergy Institute, Mount Sinai School of Medicine, New York, NY

Topic: Peanut Allergy: New Insights with Practical Implications for Diagnosis and Management

Objectives:

- *To understand new epidemiologic features of peanut allergy*
- *To apply current research findings to the diagnosis, prevention, and treatment of peanut allergy*
- *To understand current approaches toward improved therapy of peanut allergy*

0910-0940 Speaker: Dr. Deena Mandell, Wilfrid Laurier University, Waterloo, ON

Topic: "Anaphylaxis How Do They Live with It? "Objectives:

- *To present the perspective of families on the challenges of living with anaphylaxis*
- *To identify what families find most and least helpful for managing safety*
- *To help physicians understand how they can help families manage issues of safety*

0940-1000 Speaker: Dr. Shideh Mofidi, Jaffe Food Allergy Institute, Mount Sinai School of Medicine, New York, NY

Topic: Nutritional Management of Food Allergies

Objectives:

- *To identify practical approaches to food allergen avoidance and to recognize the role of diet in the treatment of food allergy*
- *To understand the impact of food allergen avoidance on growth and nutritional adequacy of the diet*
- *To review basic principles of feeding in infants and children with food allergies*

1000-1010 Discussion Period

0900-1000 NREP SESSION - Hochelaga 4

1010-1030 REFRESHMENT BREAK AMONG THE EXHIBITORS - Hochelaga 1-3

1030-1200 PRIMARY CARE/ALLIED HEALTH SYMPOSIUM - St-Maurice

SLEEP DISORDERS - THE TROJAN HORSE OF ATOPY

Chairs: Ms. J. Gillespie and Dr. Charles Frankish

1030-1055 Speaker: Dr. Robert Brouillette, Montréal Children's Hospital, Montréal, QC

Topic: Sleep Disorders in the Pediatric Population Where It Starts and Should Stop!

Objectives:

- *To recognize sleep disorders in children using easily accessible diagnostic tools*
- *To understand causes of and treatment options for sleep disorders and to be aware of outcomes in untreated pediatric sleep disorders*
- *To understand the immunologic aspects of sleep disorders*

1055-1120 Speaker: Dr. John Kimoff, Royal Victoria Hospital, Montréal, QC

Topic: Snoring in Adulthood: Much More than a Lousy Bed Partner

Objectives:

- *To recognize and assess adults with sleep disorders using easily accessible diagnostic tools*
- *To understand the importance of diagnosis of sleep disorders and to be aware of treatment options*
- *To recognize how sleep disorders affect the immune system*

1120-1145 Speaker: Dr. Najib Ayas, Vancouver General Hospital, Vancouver, BC

Topic: The Public Health and Economic Impact of Sleep Apnea

Objectives:

- *To understand the public health and economic implications of sleep apnea*
- *To understand the cost-effectiveness of therapy for sleep apnea*

1145-1200 Panel Discussion

1030-1225 THE JEFFREY MODELL CANADIAN IMMUNODEFICIENCY NETWORK SYMPOSIUM - Grand Salon

Chair: Dr. Bruce Mazer

1030-1040 Speaker: Dr. Adelle Atkinson, The Hospital for Sick Children, Toronto, ON

Topic: Overview of the Network and Its Goals

1040-1110 Speaker: Dr. Elie Haddad, Hôpital Sainte Justine, Montréal, QC

Topic: "Wiskott-Aldrich Syndrome: A Disease of the Cytoskeleton. What Does It Mean?"

Objectives:

- *To describe the role of Wiskott-Aldrich syndrome protein (WASP) in the rearrangement of the cytoskeleton*
- *To understand the role of the cytoskeleton in the homeostasis of the immune system*
- *To understand how abnormal or absent WASP may induce the biological and clinical Wiskott-Aldrich Syndrome phenotype*

1110-1140 Speaker: Dr. Stuart Turvey, BC Children's Hospital, Vancouver, BC

Topic: "Clinical Application of Molecular Testing for Primary Immune Deficiency"

Objectives:

- *To understand the presentation of primary immune deficiency*
- *To understand the diagnostic strategies in primary immune deficiency*
- *To appreciate the benefits of molecular diagnosis in primary immune deficiency*

1140-1210 Speaker: Dr. Christine McCusker, Montréal Children's Hospital, Montréal, QC

Topic: "Home Immune Globulin Therapies"

Objectives:

- *To describe implementation of home therapy*
- *To discuss its advantages and disadvantages*
- *To define available treatment options*

1210-1225 Discussion Period

1215-1330 LUNCHEON SYMPOSIUM* - Marquette/Jolliet

Sponsored by GlaxoSmithKline

Speaker: Dr. Frederick Hargreave, Firestone Institute of Respiratory Health, Hamilton, ON

Topic: "Using Sputum or NO to Guide Treatment of Airway Inflammation"

Chair: Dr. Richard Warrington

1330-1515 PLENARY SESSION II - Grand Salon

"Advances in Autoimmune Diseases, Allergy, and Immune Regulation"

Chairs: Drs. Judah Denburg and Dean Befus

1330-1410 Speaker: Dr. John Gordon, University of Saskatchewan. Saskatoon, SK

Topic: "Tolerogenic Dendritic Cells as a Therapeutic Tool in Allergy"

Objectives:

- *To review immune regulation in asthma*
- *To understand the potential for immunologic tolerization of asthma pathophysiology*

1410-1420 Discussion Period

1420-1500 Speaker: Dr. Kelly McNagny, University of British Columbia, Vancouver, BC

Topic: "Stem Cells and Allergies"

Objectives:

- *Role of mast cell and eosinophil trafficking in autoimmune diseases*
- *Role of CD34 in mast cell and eosinophil function*
- *Role of CD34 proteins in cell fats*

1500-1515 Discussion Period

1515-1530 REFRESHMENT BREAK AMONG THE EXHIBITORS - Hochelaga 1-3

1530-1700 PLENARY SESSION II - continued - Grand Salon

1530-1610 Speaker: Dr. Kathy Siminovitch, Mount Sinai Hospital, Toronto, ON

Topic: "Molecular Cascades and Cell Signaling in Autoimmunity"

Objectives:

- *To outline major strategies for identifying gene variants underlying common diseases*
- *To describe advances in the genetic dissection of several common autoimmune diseases*
- *To demonstrate the utility of genetic data in unraveling the molecular pathophysiology of autoimmune disease*

1610-1650 Speaker: Dr. Robert Inman, Toronto Western Hospital, Toronto, ON

Topic: "Recent Advances in Mechanisms and Therapeutic Interventions in Spondyloarthritis"

Objectives:

- *To understand the new concepts in microbes and arthritis*
- *To update new therapies for spondyloarthritis*

1650-1700 Discussion Period

1700-1730 NREP SESSION - Saguenay

1700-1800 RCPSC ALLERGY AND CLINICAL IMMUNOLOGY SPECIALTY COMMITTEE MEETING (Closed) - Matapedia

Chair: Dr. Donald Stark

CAAIF FUNDRAISING DINNER AND SILENT AUCTION

Le Windsor - Versailles/Windsor Ballrooms

1900-2400

Saturday, October 28, 2006

0700-1430 REGISTRATION - Mezzanine

0800-1430 VISIT EXHIBITS - Hochelaga 1-3

0815-0930 PLENARY BREAKFAST SYMPOSIUM* - Marquette/Jolliet

Sponsored by Schering Canada

Speaker: Dr. Philippe Devillier, Hôpital Foch, Suresnes, France

Topic: Comparative Pharmacologic Profiles of Antihistamines

Chair: Dr. Eric Leith

0930-1030 INTEREST SECTION BREAKOUT SESSIONS (Please check at the on-site CSACI registration desk for room assignments)

ALLIED HEALTH "CSI MONTRÉAL"

Chairs: Ms. J. Gillespie and Dr. Michael Mandl

Discussion of the clinical, scientific, and investigation/management of active case files in cases submitted by the membership.

ASTHMA

Chair: Dr. Harold Kim

ANAPHYLAXIS

Chair: Dr. David Fischer

IMMUNOLOGY

Chair: Dr. Bruce Mazer

PEDIATRICS

Chair: Dr. Timothy Vander Leek

RHINOSINUSITIS

Chair: Dr. Paul Keith

1030-1100 REFRESHMENT BREAK AMONG THE EXHIBITORS - Hochelaga 1-3

1100-1130 ANNUAL GENERAL MEEETING - CSACI (MEMBERS ONLY) - Grand Salon

Chairs: Drs. Susan Waserman and Richard Warrington

1130-1200 ANNUAL GENERAL MEETING - CAAIF (MEMBERS ONLY) - Grand Salon

Chairs: Drs. Eric Leith and Allan Becker

1215-1330 PLENARY LUNCHEON SYMPOSIUM* - Marquette/Jolliet

Sponsored by AstraZeneca

Pro-Con Debate

Dr. Charles Chan, University Health Network, Toronto, ON

Dr. Pierre Ernst, McGill University Health Centre, Montréal, QC

Topic: Timely Escalation of Maintenance Therapy Will Achieve Better Asthma Outcomes

Chair: Dr. Susan Waserman

FREE AFTERNOON

1415-1515 CSACI JOURNAL MEETING (closed) (Room to be confirmed)

1730-1930 LIBATION AMONGST THE POSTERS - Grand Salon

Chairs: Drs. Dean Befus, Richard Warrington, Kent HayGlass, and Stuart Carr

CSACI ANNUAL DINNER AND
AWARDS PRESENTATION
1930-2200
Marquette/Jolliet
1930 **DINNER**
2100 **AWARDS PRESENTATION**

Sunday, October 29, 2006

0730-1100 VISIT EXHIBITS - Hochelaga 1-3

0730-0900 PLENARY BREAKFAST SYMPOSIUM* - Marquette/Jolliet

Sponsored by Merck Frosst Canada Inc.

Speaker: Dr. Keith Payton, London Health Sciences Centre, London, ON

Topic: The Role of Rhinitis in Asthma

Chair: Dr. Robert Schellenberg

0900-1030 PLENARY SESSION III - OCCUPATIONAL ALLERGY SYMPOSIUM

Grand Salon

Chairs: Drs. Eric Leith and Charles Frankish

0900-0940 Speaker: Dr. Catherine Lemiàre, Hôpital du Sacré-Coeur, Montréal, QC

Topic: Airway Inflammation and Occupational Asthma: Insight from Induced Sputum

Objectives:

- *To be aware of the type of airway inflammation seen in occupational asthma*
- *To understand the importance of assessing airway inflammation in the investigation of occupational asthma*
- *To be able to recognize occupational eosinophilic bronchitis*

0940-1020 Speaker: Dr. André Cartier, Hôpital du Sacré-Cour, Montréal, QC

Topic: "Update of Clinical Aspects of Occupational Lung Diseases"

Objectives:

- *To provide update on new etiologies of occupational asthma*

- *To provide update on risk factors for occupational asthma*
- *To provide update on the investigation of occupational asthma*

1020-1030 Discussion Period

1030-1100 REFRESHMENT BREAK AMONG THE EXHIBITORS - Hochelaga 1-3

1100-1230 PLENARY SESSION III - continued - Grand Salon

1100-1140 Speaker: Dr. Denis Sasseville, Royal Victoria Hospital, Montréal, QC

Topic: "Occupational Skin Diseases"

Objectives:

- *To recognize irritant and allergic occupational contact dermatitis*
- *To develop a systematic approach to diagnosis*
- *To understand the principles and techniques of patch testing in contact dermatitis*

1140-1220 Speaker: Dr. J. David Miller, Carleton University, Ottawa, ON

Topic: "Population Health Aspects of Mould in Non-Industrial Workplaces and Residences"

Objectives:

- *To provide an explanation of the advice from Health Canada on mould and health*
- *To review areas of consensus on the population health effects of mould between Health Canada, the World Health Organization, and the US National Academy of Sciences panel reports on asthma (2000) and building dampness and health (2004)*

1220-1230 Discussion Period

1230-1245 CLOSING REMARKS BY CSACI PRESIDENT - Grand Salon

Dr. Charles Frankish

Note: Program is subject to change

***Breakfast and luncheon symposia are nonaccredited sessions**

Mission Statement

The mission of the Canadian Society of Allergy and Clinical Immunology is the advancement of the knowledge and practice of allergy, clinical immunology, and asthma for optimal patient care. The Society is also dedicated to improving the quality of life of people with allergies through research, advocacy, and continued professional and public education.

CPD COMMITTEE

Dr. Charles Frankish (Chair)
Dr. Zave Chad
Dr. Keith Payton
Dr. Susan Waserman

Annual Scientific Program Committee 2005

Dr. Robert Schellenberg (Chair)
Dr. Susan Waserman
Dr. Allan Becker
Dr. Dean Befus
Dr. Zave Chad
Dr. Judah Denburg
Dr. Charles Frankish
Ms. Jo-Anna Gillespie
Dr. Kent T. HayGlass
Dr. Redwan Moqbel
Dr. Richard John Warrington

2007 Csaci Annual Scientific Meeting Edmonton, Alberta September 27TH To 30TH, 2007 Edmonton Festival City

Check out our Web site for future developments

2006 Awards Recipients

- Bram Rose Memorial Lectureship - Dr. Scott Sicherer
- David McCourtie Memorial Lectureship - Dr. André Cartier
- CSACI Award for Research in Immunology - Dr. Kathy Siminovitch
- The Jerry Dolovich Award - Dr. Zave Chad
- The Royal College Speaker - Dr. Robert Inman

The Csaci and Caaif Gratefully Acknowledge the Following Companies For Their Generous Support: Platinum Sponsors

ALTANA PHARMA
ASTRAZENECA
GLAXOSMITHKLINE
MERCK FROSST
NOVARTIS

Gold Sponsors

SCHERING CANADA INC.

Silver Sponsors

TALECRIS BIOTHERAPEUTICS

Bronze Sponsor

ROSS LABORATORIES

Article Categories:

- Annual Scientific Meeting

Allergy, Asthma, and Clinical Immunology
Volume 02, Issue 04, December 2006, Pages 138-163
Annual Scientific Meeting

Meeting Abstracts

Annual Scientific Meeting, Montreal, October 26-29, 2006

Digital Ischemia Related to Gemcitabine

R. Khouqeer, M. Almarzouqi, M. Baron

Introduction: We present the occurrence of gemcitabine-induced digital ischemia in a 73-year-old female with a history of breast cancer metastatic to the peritoneum and the lung. She presented with painful purple fingers after 10 days of her second cycle of gemcitabine. She had no prior history of Raynaud's or any vascular diseases. *Case Report:* A 73-year-old female was diagnosed with breast cancer in May of 1992, for which she was treated with adjuvant chemotherapy (cyclophosphamide and Adriamycin), radiation, and then surgical resection. In addition to that she received tamoxifen for 5 years. However, in the autumn of 2000, she was diagnosed with infiltrating adenocarcinoma carcinoma of the omentum consistent with metastases from the breast and therefore was placed back on tamoxifen. She failed this therapy, as evidenced by recurrent ascites, and she was started on navelbine for a total of eight cycles, with an excellent response. However, in November 2001 her disease appeared to have progressed, with evidence of increased ascites. Her chemotherapy was stopped, and she was started on Arimidex. The ascites resolved. She had progressive disease in May 2003 and therefore was switched to Megace. Then in October 2003 she was switched to gemcitabine, which was tolerated well during the first and second cycles. After 10 days of the second cycle she started to develop a bluish discoloration of all her fingers, which was associated with severe pain. She had normal pulses and no ischemic changes in her feet. Her past medical history included DM2, asthma, A.fib, pneumonias, and no history of blood transfusion or any liver disease. She is a nonsmoker and does not consume any alcohol. The physical examination of her fingers revealed good radial and ulnar pulses. The physical examination also revealed blue fingertips (all 10) and severe tenderness, with small ulcers on her fingers. Laboratory investigations: normal CBC, LFT, renal, ESR 115, CRP 49, +ANA (Spk)1:80, -ve ACLA, ANCA, and Cryo and normal LAC and C3, C4. ECHO showed normal EF, MR, TR, PAP 41. An angiogram showed slow distal filling with very slight improvement with nitro challenge. In hospital she was started on ASA and nifedipine XL for 1 week, with little response. She was put on Solu-Medrol 60 mg IV for 1 week and then switched to oral prednisone 50 mg Q wk, which was tapered down to 5 mg Q wk over 11 weeks, with a good response that was apparent within 2 weeks after initiating the corticosteroids. *Conclusion:* This case illustrates that gemcitabine alone may produce digital ischemia. This is only the third case in which this has been reported to occur in patients not on other chemotherapeutic agents as well.

Management of Anaphylaxis in a Tertiary Care Emergency Department

Anne K. Ellis, James H. Day

Background: Publications regarding the emergency management of anaphylaxis report rates of epinephrine administration between 16 and 57%; EpiPen prescription rates vary from 0 to 22%. *Objective:* To document treatment, observation, and discharge strategies for patients with anaphylaxis in a tertiary care emergency department (ED) in Canada. *Methods:* Retrospective chart review of ED visits from 1999 to 2002; all charts with a discharge diagnosis of "anaphylaxis" or "allergic reaction" were evaluated; if symptoms/signs were consistent with anaphylaxis included in the study. Management strategies, total observation time, and discharge management/advice were analyzed descriptively and compared to symptomatology via chi-square. *Results:* 129 patients were treated for an acute systemic allergic reaction consistent with anaphylaxis in the period reviewed. Average age was 31.8 years (range 0.9-79), and 62 (48.0%) were female. Details of management strategies are presented in the Table.

The overall rate of epinephrine use was 79.1%, but it was given as first-line therapy in only 66 (51.1%) and was given subcutaneously (SC) by ED personnel in all cases. Documented hypotension and/or decreased SaO_2 resulted in higher rates of corticosteroid administration ($p < .034$). Pediatric cases (≤12 yr) were significantly less likely to receive corticosteroids and H_2 antagonists ($p < .025$), and trended toward decreased epinephrine use ($p = .08$).

Average ED observation time was 3.8 hours. Nineteen patients had a documented biphasic reaction (13 were discharged from the ED prior to the second phase); 4 were subsequently admitted (total admission rate 8.4%). *Conclusions:* Anaphylaxis was treated with epinephrine by the ED at a higher rate than reported elsewhere but was not routinely administered first line and was given SC, rather than IM, discordant with published guidelines. Anaphylaxis was more likely to be labeled an "allergic reaction," leading to underreporting, and EpiPens remained underprescribed, although more frequently than reported elsewhere. Average observation time was 4 hours, which was insufficient for 13 patients (10%) who returned after discharge from the ED with biphasic reactivity. Despite

improvements, anaphylaxis continues to be an underdiagnosed and under-treated condition. *Support:* This study was internally funded.

Allergen-Induced Changes in Adhesion Molecule Expression and Function on Bone Marrow Progenitor Cells in Asthmatic Subjects

A. Catalli, J. Thomson, I. Babirad, M. Duong, R. Watson, G. Gauvreau, P.M. O'Byrne, R. Sehmi, McMaster University and St. Joseph's Healthcare, Hamilton, ON

Rationale: Eosinophilic airway inflammation is an underlying feature of asthma and is associated with activation of hematopoietic events in the bone marrow. Understanding the mechanisms regulating progenitor cell mobilization and trafficking from the bone marrow into the airways may be important for the development of effective asthma treatment. *Objectives:* We set out to characterize preferential activation of adhesion pathways for the mobilization of hematopoietic progenitor cells from the bone marrow. *Methods:* Bone marrow aspirates were obtained from mild asthmatics pre- and several time points post-allergen challenge. Low-density nonadherent mononuclear cells were harvested by density gradient centrifugation on Percoll. Changes in expression of β1 (CD49d/α4β1, CD49e/α5β1) and β2 (CD11b/Mac-1) integrins on $CD34^+45^+$ progenitor cells were assessed by flow cytometry. A sequential multigating strategy was used to enumerate level and intensity of expression. Adhesion of an enriched $CD34^+$ cell population to fibronectin (20 μg/mL coated wells) following SDF-1 stimulation was similarly enumerated by flow cytometry. *Results:* There was a significant decline in CD49d levels on $CD34^+45^+$ cells 24 hr post-allergen challenge as compared to baseline (pre-Ag: 10.7 ± 3.8 vs post-Ag; 3.0 ± 0.9, $p = .02$, $n = 9$ and 13, respectively), which had begun to recover by 48 hrs. There was no significant allergen-induced change in CD49e or CD11b expression (CD49e: 34.8 ± 3.4 vs 32.4 ± 7.0, $n = 13$ and CD11b: 4.1 ± 1.1 vs 2.7 ± 1.0, $n = 14$ in pre vs post, respectively). Adhesion to fibronectin was attenuated 24 hr post-allergen challenge, corresponding to the decline in CD49d expression, and had begun to recover by 72 hrs. *Conclusion:* Our data suggest that down-regulation of β1 integrin expression, as well as the associated adhesive properties of progenitor cells, may be responsible for reducing the tethering forces to extracellular matrix components such as fibronectin within the bone marrow, thus facilitating the egress of $CD34^+45^+$ cells to the peripheral circulation during an allergic inflammatory response. *Support:* This abstract is funded by the Canadian Institutes for Health Research and Father Sean O'Sullivan Research Council.

Family Asthma Program© Parent and Caregiver Objective Outcomes

N.L. Ross, C.A. Gillespie, B.A. Kulbaba, S.E. Filuk, L.J. Stewart, J.C. St. Vincent, D.L. Stockwell, N.F. Cisneros, A.B. Becker, The Children's Asthma Education Centre, University of Manitoba, Winnipeg, MB

The Children's Asthma Education Centre (CAEC) evaluated the effectiveness of our Family Asthma Program© (FAP) in meeting objectives of asthma education. Current guidelines for asthma education recommend that programs be evaluated. Asthma education objectives listed in *The Canadian Network for Asthma Care Asthma Educator's Guide* are to improve patients' asthma knowledge and skills; improve patients' self-assessment abilities/skills; improve patients/caregivers' self-confidence; improve patients' adherence to behaviours that promote asthma self-management; and improve the relationships between patients and health care professionals. We evaluated 98 families of children ages 3 to 11 years from our FAP from April 2005 to May 2006. During the first session families are given a questionnaire and asked whether they agree, disagree or are unsure about the following four statements: *I feel comfortable asking my doctor about asthma*; *I feel confident I can manage my child's asthma*; *When my child has an asthma attack I know what to do*; *I know when and how to give my child's asthma medicine*. After the final session the families completed the same questionnaire.

Conclusion: The CAEC FAP effectively meets objectives for asthma education. There is significant improvement in the families' knowledge and confidence in managing their child's asthma even among parents who already felt comfortable about discussing asthma with their physician.

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

A.K. Ellis, L. Crawford, J.A. Denburg, Division of Clinical Immunology and Allergy, Department of Medicine, McMaster University, Hamilton, ON

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-5Rα, as surrogate molecular markers of Eo/B differentiation. These same methods have yet to be established in peripheral blood (PB)

samples. *Objective:* To use Q-PCR to determine the kinetic patterns of expression of CB Eo/B-lineage specific genes in PB 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 72 hours post-stimulation, RNA was isolated, reverse transcribed, and expression of IL-5Rα, GATA-1, and MBP was determined using comparative Q-PCR in a multiplex reaction. Relative expression ratios of stimulated to unstimulated cells were calculated using the delta-delta Ct method. *Results:* Stimulation of PB NANMC with IL-5 resulted in an upregulation of GATA-1 expression, peaking at 24 hours, with a slower return to baseline expression than that observed in CB. MBP expression was minimally altered at all time points, compared with CB, where slow upregulation, maximal at 72 hours, had been observed. There was completely stable expression IL-5Rα, 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 in part by the AllerGen/Bayer/CAAIF Immunodeficiency and Immunomodulation of Allergic Inflammation Clinician-Scientist Research Fellowship.

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

R. Amin, S. Balkovec, F. Ratjen, P. Subbarao, The Hospital for Sick Children and The University of Toronto, Toronto, ON

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 to 120 mL in increasing increments of 20 mL. For each tidal volume, EBC was collected over 20-minute periods for respiratory rates ranging from 20 to 70 breaths per minute in increments of 10 breaths/min. The 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 were analyzed using factorial ANOVA and linear regression. *Results:* EBC volume ranged from 0.37 to 3.8 mL. Using factorial ANOVA, both tidal volume and respiratory rate were found to be significant factors in determining EBC volume ($p < .05$). A trend toward significance was observed for the sleeve temperature ($p = .053$). EBC volume had the strongest correlation with tidal volume ($r = .74$, $p < .05$) followed by respiratory rate ($r = .13$, $p = .4$). 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.

Alternaria Allergy and Fall Asthma Admissions

C.A. Gillespie, S.E. Filuk, N.L. Ross, B.A. Kulbaba, L.J. Stewart, W.T.A. Watson, A.B. Becker, The Children's Asthma Education Centre, Children's Hospital, Winnipeg, MB

Background: Fall is the peak season for asthma exacerbations. *Alternaria* levels increase in prairie agricultural regions in the fall. At the Children's Hospital in Winnipeg, as a small pilot project, we asked whether children who were admitted to hospital in fall were more likely to be allergic to *Alternaria* than children admitted in winter. *Methods:* Families of children ≥3 years old admitted to hospital for asthma in September and October 2004 ($n = 67$) and December 2004 through February 2005 ($n = 28$) were approached during or following admission for study participation. Children were excluded if they lived outside Winnipeg, had additional respiratory conditions, or were deemed otherwise unsuitable. Following written consent, a questionnaire to gather pertinent history was completed, children were skin-tested to common aeroallergens, including *Alternaria*, and spirometry was performed when possible. *Results:* Participation included a total of 15 children, with 1 child being admitted in both seasons. Although nonsignificant, results indicated a trend toward a positive relationship between *Alternaria* allergy and fall admissions.

Conclusions: Due to the small number of participants it is difficult to make any definite conclusions. However, health care providers and asthma educators should be aware of the trend, especially in prairie regions. Appropriate management plans and education may help lower the admission risk for *Alternaria*-sensitive individuals.

Regulation of Progenitor Cell Traffic in Allergic Inflammatory Responses: Stromal Cell-Derived Factor 1-Stimulated Adhesive Responses of $Cd34^+$ Cells

Jennifer V. Thomson, Irene M. Babirad, Roma Sehmi, Firestone Institute for Respiratory Health, McMaster University, Hamilton, ON

Stromal cell-derived factor 1α (SDF-1; CXCL12) is a potent progenitor cell chemoattractant and is thought to retain $CD34^+$ progenitor cells within hematopoietic compartments, such as the bone marrow (BM). Ligation of SDF-1α coupling to its exclusive receptor, CXCR4, results in mobilization of $CD34^+$ cells to the peripheral circulation (PB). Little, however, is known about the interplay of chemokines in mediating the egress of $CD34^+$ cells from the BM in allergic inflammation. We have previously shown that 24-hour post-allergen (Ag) challenge in dual-responder asthmatics, BM $CD34^+$ cells showed significant attenuation in CXCR4 expression and reduced migrational responsiveness to SDF-1α in vitro. This was associated with a significant increase in the proportion of $CD34^+$ cells expressing CXCR4 in PB. We propose that increased mobilization of progenitor cells may reflect changes in SDF-1α-stimulated adhesive interactions of progenitor cells with BM extracellular matrix components. In the current study we investigated modulation of adhesive interactions of progenitors to fibronectin in vitro. We found that SDF-1α stimulated adhesion of $CD34^+$ progenitor cells to fibronectin in a dose-dependent manner optimal at 10 ng/mL ($p < .05$). The adherence of $CD34^+$ cells was significantly inhibited by anti-VLA4 > anti-VLA-5 > anti-VLA4 + VLA-5 (69 ± 13%, 59 ± 28%, 58 ± 29% inhibition, respectively; $p < .05$). Pre-exposure to CCR5-acting proinflammatory chemokines such as RANTES (200 ng/mL) and MIP-1α (20 ng/Ml) inhibited SDF-1α-stimulated adhesion of $CD34^+$ cells to fibronectin (82 ± 12%, 60 ± 32% inhibition, respectively, $p < .05$). Our findings show that SDF-1α maintains the retentivity of progenitor cells to the bone marrow through adhesion to fibronectin and attenuation of this adhesive response may promote the egress of progenitor cells to the PB in an allergic inflammatory response. This work was supported by the Canadian Institutes for Health Research and the Ontario Thoracic Society.

Asthma Care Maps Are a Valuable Tool in the Management of Asthma

Peter Strachan, Cathy Gillespie, Leslie Galloway, Nancy Ross, Allan Becker, Section of Allergy and Clinical Immunology, Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, MB

Background: Patient education is an important underpinning of asthma care. The Children's Asthma Education Centre (CAEC) provides asthma education for children with asthma and their families. However, many children with asthma and their families do not get referred for asthma education. Children's Hospital recently introduced an in-patient care map for children with an asthma exacerbation. *Hypothesis:* Introduction of an in-patient asthma care map increases rates of referral for asthma education. *Methodology:* Retrospective analysis of medical records for children admitted to hospital in September 2000 (before introduction of the care map) and September 2005 (after introduction) was carried out. We assessed (1) proportion of children referred to CAEC, (2) duration of admission, (3) pulmonary function testing, (4) discharge use of inhaled corticosteroids, and (5) repeat emergency department visits within 3 months. *Results:* There was a highly significant increase from 13 of 26 children referred to CAEC in 2000 to 24 of 24 children in 2005 ($p < .0001$). The care map was also associated with a significant increase in PFTs (from 7 to 76%). Significantly more children were discharged using inhaled corticosteroids (85 vs 100%) and there was a reduction in subsequent ER visits from 31 to 17%. Also, the average length of admission decreased from 59 hours in 2000 to 39 hours in 2005 ($p < .0001$). *Conclusion:* An asthma care map results in significantly more asthma education for children admitted to hospital with an asthma exacerbation. In addition, the care map was associated with shorter duration of hospitalization and decreased likelihood of subsequent emergency department visits.

Hereditary Angioedema: Literature Review and a Case Report

Farag I. Farag, Ashraf A. Abdel-Naby, Hanan Taha, Amgad Abd-Ellatief, Faculty of Medicine, Suez Canal University, Egypt; International Medical Center, Egypt; Holding Company of Biological Products and Vaccines (VACSERA), Egypt; Omega Laboratories Limited, Canada

Hereditary angioedema (HAE) is a rare genetic disease of the complement system manifesting as recurrent attacks of massive edema of the skin and respiratory and gastrointestinal systems. The basic defect in HAE is the deficiency or dysfunction of the C1-esterase inhibitor (C1-INH). We report here a case of HAE that was misdiagnosed as a combination of intractable asthma and skin allergy for 30 years until successfully diagnosed and treated at VACSERA and the International Medical Center in Egypt. *Subject and Methods:* The case was a 48-year-old male with positive consanguinity and a family history suffering since 2002 from severe recurrent episodes (7 days/month) of a wheezy chest, shortness of breath, and edema of the face, neck, and body with dysphagia, constipation, and abdominal cramps. Antihistamines, bronchodilators, and megadoses of corticosteroids were ineffective in aborting the attacks. Onset started at age 16; it was mild at first and then worsened with age. C4 and C1-INH serum levels during episodes were

2 mg/dL (normal 10-40) and 9 mg/dL (normal 15-35), establishing the diagnosis of HAE. Marked clinical improvement and elevation of C4 and C1-INH levels were detected when the patient was maintained on danazol (600 mg/day). *Conclusions:* We conclude that HAE, albeit rare, should be included in the differential diagnosis of intractable asthma associated with skin allergy.

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

Sari M. Herman, Stephen Betschel, Gordon Sussman, Department of Medicine, McMaster University, Hamilton, ON; Division of Clinical Immunology and Allergy, Department of Medicine, University of Toronto, Toronto, ON

Purpose: To report an unusual case of hemolytic anemia following intravenous immunoglobulin (IVIG) administration. We review the published literature examining hemolytic anemia with the use of 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 7-year history of chronic urticaria refractory to conventional therapy who was treated with IVIG. *Results:* A 3-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 (hemoglobin 106 g/L) with polychromasia and a few spherocytes present, hyperbilirubinemia (total bilirubin 79 μmol/L), and a low haptoglobin (<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 2 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 hemoglobin levels with IVIG therapy is recommended.

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

J.L.P. Protudjer, G.D.M. Marchessault, C. Benoit, A.L. Kozyrskyj, A.B. Becker, John Buhler Research Centre, Winnipeg, MB

Rationale: Body image is important to preadolescents. 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 that non-asthmatics. *Methods:* Children enrolled in a cohort study born in Manitoba in 1995 were assessed by pediatric allergists for asthma at age 8 to 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 = .69) in body dissatisfaction in asthmatic versus non-asthmatic children. Neither asthmatic boys and girls (p = .22) nor non-asthmatic boys and girls (p = .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 = .91), muscle (p = .44), weight (p = .37), or height (p = .48) dissatisfaction were found between asthmatics and non-asthmatics. *Conclusions:* Asthma does not affect body dissatisfaction in preadolescent children. Based on other literature, we speculate that other sociocultural factors, such as peer pressure and media, and biologic factors, such as puberty, influence body dissatisfaction in preadolescent 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.

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, BC

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; therefore, we examined the role of 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 bronchoalveolar lavage (BAL) cells. Histologic 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 evalulated via in vitro migration assays. We found that CD34KO mice had far fewer cells in BAL than WT controls (1.162 ± 0.32 compared with 2.946 ± 0.417 × 10^6 cells/mL in WT; p = .0015). All hematopoietic subsets were significantly reduced, and histologic 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 = .03; mast cell counts: 6.28 ± 1.5/section vs 18.17 ± 1.5 in WT, p = .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 toward eotaxin in an in vitro migration assay compared with WT eosinophils. Taken together our results suggest that 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.

Is There a Correlation Between Skin-Prick Tests and Bronchial Hyperresponsiveness in Children?

J.J. Liem, R. Rabbani, A.L. Kozyrskyj, A.B. Becker, Section of Allergy and Clinical Immunology, Department of Pediatrics and Child Health, University of Manitoba; The Manitoba Institute of Child Health, Department of The Children's Hospital Foundation, University of Manitoba; Departments of Community Health Sciences and Pediatrics and Child Health, Faculties of Medicine and Pharmacy, University of Manitoba, Winnipeg, MB

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 was 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). Four 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 < .0001) and -0.310 (p < .0001), respectively. When stratified by gender, the correlation for boys was -0.310 (p < .0001) and -0.331 (p < .0001) for the number of skin-prick tests and total mean wheal diameters, respectively. For girls, the correlation was lower at -0.260 (p < .0001) and -0.269 (p < .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.

Likelihood of Adrenaline Auto-Injector Prescriptions in Food Allergy Patients

D.A. Fischer, H.L. Kim, Barrie, ON, Kitchener, ON, University of Western Ontario, London, ON

Introduction: Adrenaline is the drug of choice for anaphylactic food allergies. Many patients are neither referred to an allergist nor prescribed an adrenaline auto-injector (AAI) despite a suitable history. We examine the likelihood of primary care providers prescribing AAIs in patients with eventually confirmed food allergy. *Methods:* All food allergy patients in two office-based practices were prospectively assessed over a 7-month period. All patients with a history compatible with an IgE-mediated food reaction and a confirmatory positive skin-prick test were included. The percentage of patients having an AAI prescribed by the referring physician was calculated. Also, the percentage of AAIs prescribed based on age and the food allergen involved were calculated. The food allergy categories were peanut, tree nuts, egg, milk, crustacea, and miscellaneous. The percentage of AAIs prescribed in each centre was calculated. *Results:* The likelihood of an AAI being prescribed to any patient was 80 in 182 (44%). In Barrie 44 of 85 (51.8%) and in Kitchener 36 of 97 (37.1%) had AAIs prescribed. For individual allergens, the results were milk, 2 of 13 (15.4%); egg, 10 of 30 (33.3%); peanut, 42 of 81 (51.9%); tree nuts, 17 of 36 (47.2%); crustacea, 11 of 24 (45.8%); and miscellaneous, 6 of 18 (33.3%). When stratified for age, the results were age < 2, 13 of 46 (28.2%); age 2 to 5, 24 of 44 (54.5%); age 6 to 16, 17 of 38 (44.7%), age > 16, 26 of 54 (48.1%). *Conclusions:* Patients with anaphylactic food allergies were underprescribed AAIs. Patients with milk and egg allergies were less likely to have an AAI prescribed. Primary care providers were less likely to prescribe auto-injectors to very young children. Physician education is required to increase prescriptions for AAIs for appropriate patients.

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

M. Duong, P. Subbarao, E. Adelroth, G. Obminski, T. Strinich, M.D. Inman, S. Pedersen, P.M. O'Byrne, McMaster University, Hamilton, ON; University of Toronto, Toronto, ON; Umea University, Umea, Sweden; University 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 were examined. *Methods:* Twenty-six steroid-naïve asthmatics with EIB were randomized to two 3-week periods of low (40 or 80 μg) and high-dose (160 or 320 μg) inhaled ciclesonide in a double-blind crossover trial, with two parallel arms and a washout period of 3 to 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 ≥5% were found in 10 subjects. Sputum eosinophils were significantly correlated to EIB severity (Spearman's rho -0.61; $p < .01$). Sputum eosinophil count was also a significant predictor of the temporal response of EIB to high- (160 and 320 μg) but not low-dose (40 and 80 μg) ciclesonide therapy. The latter was characterized 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.

Efflux of Peripheral Blood Basophils after Allergen Inhalation by Mild Asthmatics Using Cell Surface Staining with an Antibody to CD203C

M.Y. Evans, E.J. Farrell, T.X. Strinich, A.J. Baatjes, M.Y. Duong, G.M Gauvreau, McMaster University, Hamilton, ON

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 and 0.5, 2, 4, 6, and 24 hours after allergen and diluent inhalation from eight mild asthmatic subjects. Basophils were stained by three methods: (a) direct surface immunostaining with anti-CD203c (Immunotech) and enumeration by flow cytometry; (b) indirect surface immunostaining 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 ($p < .05$), with a maximal decrease occurring at 2 hours, and complete resolution by 24 hours post-AC. There was also a decrease in PB basophils after AC when compared to diluent when staining with CD203c ($p < .05$), with a maximal decrease at 6 hours and complete resolution by 24 hours. We did not observe a decrease in PB basophils after AC compared to diluent using the BD antibody cocktail or alcian blue ($p > .05$). *Conclusion:* We observed efflux of basophils from PB of asthmatic subjects after inhaled allergen challenge using direct immunostaining of PB basophils with anti-CD203c.

Socio-Economic Factors Related to Asthma Control in Children

Shannon Cope, Wendy Ungar, Richard Glazier, The Hospital for Sick Children, Toronto, ON

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 socio-economic 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 to 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 β2-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 health care use 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 with 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.

The Risk of Peanut Allergy in Siblings of Peanut-Allergic Children

S. Huq, J.J. Liem, J.L.P. Protudjer, A.L. Kozyrskyj, A.B. 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 2005-2006, a survey was sent to 441 households of children born in 1995 in Manitoba (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 on the index children. The likelihood (odds ratio [OR]) of peanut allergy in siblings of peanut-allergic children versus non-peanut-allergic children was determined. *Results:* 370 of 441 (83.9%) of the surveys were completed (urban, rural, and First Nations communities). Twenty-nine (7.8%) children were peanut allergic (physician diagnosis ± skin-prick test/RAST/oral challenge). Five children were sensitized but not allergic to peanut. Nine children did not have siblings. There were 43, 9, and 568 siblings of peanut-allergic, peanut-sensitized but 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 with 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.

Seasonal Variation in Onset of Urticaria.

D.A. Fischer, Barrie, ON, University of Western Ontario, London, ON

Introduction: Although most episodes of urticaria are thought to be from an immune response to the presence of a virus, many physicians still inform their patients that there is an eventually identifiable cause. We present data on onset of urticaria related to the seasons involved to identify whether there is a pattern that mimics viral infections versus random allocation. *Methods:* All urticaria patients in an office-based practice over 1 year were asked during which month their urticaria started. Results were based on a November 2004 to February 2006 survey. Patients were included starting in January 2004, but because of increased waiting-list time data from early 2006 were also included, adjusted to reflect increased wait times in late 2005. *Results:* 198 consecutive cases of idiopathic urticaria of at least 3 days' duration were assessed. Patients with subsequent chronic urticaria were also included. The data were divided into tertiles: 75 (38%) presented in first tertile (Jan-Apr), 35 (18%) in the second tertile (May-Aug), and 88 (44%) in the third tertile (Sept-Dec). The most active months were March (26) and November (32). The least active were May (6) and July (9). *Conclusions:* Urticaria of "unknown origin" are often felt to be immunologic responses to viruses, but viral serology is too unreliable to prove this. This study shows that patients present with urticaria with seasonal differences that reflect when viruses are more like to be involved (first and third tertiles). The number of cases was much worse in March and November and much improved in May and July, also suggesting a viral pattern and not random onset.

Development of an Algorithm to Better Predict Clinical Responsiveness to Peanut

Andrea Marrin, Kevin Sun, Susan Waserman, Manel Jordana, Kent HayGlass, Department of Allergy and Immunology, McMaster University, Hamilton, ON; Department of Immunology, University of Manitoba, Winnipeg, MB

Objectives: 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 (PBMCs) to generate an algorithm to better predict clinical responsiveness to peanut. *Methods:* Four 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) nonatopic 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 the Luminex multiELISA system. *Findings:* Eighty-five 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 ti 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 with C. The Th1 cytokine IFN-gamma was also increased in TP compared with C. *Relevance:* Peanut-allergic patients demonstrated an increase in Th2 cytokine expression compared with nonatopic 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 an diagnostic algorithm to be tested in patients with unknown reactivity (Group 3).

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

R.S. Vig, A.D. Befus, H. Vliagoftis

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, neurologic, and physiologic 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 with unstressed mice. *Methods:* Mouse litters underwent a daily 3-hour maternal separation on days 1 to 10 after birth, during which other litters were left unhandled. On days 31 and 36, mice were sensitized to chicken egg ovalbumin (OVA) via an IP injection with aluminum hydroxide and were later challenged intranasally (days 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 with saline-challenged animals. Interestingly, OVA challenged maternally separated males showed a significant reduction (≅50%) in BAL inflammation compared with 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-γ and interleukin (IL)-4 levels, with no change in IL-5, -6, -9, or -13. *Conclusions:* These findings suggest that neonatal stress down-regulates 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, such as alveolar size, that may be a result of early-life stress in mice.

Airway Hyperresponsiveness Predicts Increased Th1-Like Antiviral Immunity in Children with and without Asthma

R. Douville, J. Liem, Y. Li, N. Bastien, A. Becker, A. Kozyrskyj, K.T. HayGlass, CIHR National Training Program in Allergy and Asthma Research; Departments of Immunology, Medical Microbiology, and Pediatrics/Child Health, University of Manitoba; Canadian Science Center for Human and Animal Health; Department of Community Health Sciences, Faculties of Medicine and Pharmacy, Winnipeg, MB

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 that 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 hyperresponsiveness as measured by methacholine challenge (PC_{20}). *Methods:* Peripheral blood mononuclear cells (PBMC) from ≅300 children (8-9 years 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 hyperresponsiveness (PC_{20}), 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 hyperresponsiveness demonstrated increased production of Th1 cytokines and IL-10 in response to MPV compared with healthy controls (IFN-γ; $p < .05$ and IL-10; $p < .05$). Non-asthmatic children with airway hyperresponsiveness demonstrated similarly increased Th1 antiviral

responses. Weak negative correlations between antiviral IFN-γ and IL-10 responses and PC_{20} values was observed in this child cohort ($p < .01$). Concomitantly reduced CCL5 production was observed among hyperresponsive asthmatic children compared with healthy controls. Both asthmatic status and airway hyperresponsiveness contributed to this phenotype. *Conclusions:* Airway hyperresponsiveness predicts increased Th1-like antiviral 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.

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, CIHR National Training Program in Allergy and Asthma Research, Departments of Immunology, Community Health Sciences, and Pediatrics/Child Health, University of Manitoba, Winnipeg, MB

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 PBMCs from 31 atopic asthmatic and 29 healthy controls, 8- to 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%, $p = .0003$) and concomitantly reduced IFN-γ and CXCL-10 responses (median reductions 20 to 80%, p values < .0001). Second, 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 ($p < .035$ to .0001) than in control individuals. Finally, increasing acetylation resulted in a broad shift toward 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 immunologic shift toward 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 nonatopic 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. *Support:* CIHR Canada Research Chair in Immune Regulation and AllerGen. R.-C. S. holds a CIHR postdoctoral fellowship and is a trainee member of the CIHR National Training Program in Allergy and Asthma and AllerGen.

vitamin D stimulation of Bronchial Smooth Muscle Cells Induces Autocrine, Contractility, and Remodeling Processes

Yohan Bossé, Karim Maghni, Thomas J. Hudson, McGill University and Génome Québec Innovation Centre, Montréal, QC; University of Montréal, Sacré-Coeur Hospital, Research Centre, Montréal, QC

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 (BSMCs) 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 BSMCs. 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 BMSCs 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 remodeling.

Genomic and Non-Genomic Effects of Dexamethasone on Equine Peripheral Blood Neutrophils

L. Lecoq, A. Lavoie-Lamoureux, P. Vincent, J.P. Lavoie, Université de Montréal, Saint-Hyacinthe, QC

Glucocorticoids exert potent anti-inflammatory actions in a cell type-specific manner and are among the most prescribed drugs for the treatment of equine inflammatory diseases. They are believed to exert their effects primarily through interruption of cytokine-mediated pathways via gene expression (transactivation) or gene repression (transrepression). More recently, the presence of non-genomic pathways has also been described. Currently, little is known concerning the non-genomic effects of dexamethasone (DEX) on neutrophils and the requirement of glucocorticoid receptor (GR) activation for this response. The objective of this study was to evaluate the genomic and non-genomic responses of equine neutrophils to glucocorticoids and the dependency of their receptors in these processes. The genomic effects of corticosteroids were assessed by studying the IL-8, TNF-α, and the Toll-like receptor (TLR)-4 mRNA expression using real-time RT-PCR. Peripheral blood neutrophils from six healthy horses, isolated using ficoll (purity >96% and viability >98%), were incubated at 37°C, 5% CO_2 for 6 hours in the presence of 100 ng/mL LPS, and 10^{-6}M DEX alone or combined with the GR inhibitor RU486 (10^{-5} M). The non-genomic effects on neutrophils (oxidative burst) were studied using whole blood from three horses incubated in a water bath with 10^{-6} M DEX (20, 25, and 30 minutes) and 5 μM dicholorofluorescein (DCF) and then stimulated with 5 ng/mL phorbol-myristate acetate in the presence of RU486 (10^{-5} M). The oxidative burst of neutrophils was evaluated using flow cytometry. DEX significantly down-regulated the LPS-induced IL-8, TNF-α, and TLR-4 mRNA expression. Pre-treatment with DEX (25 and 30 minutes) similarly attenuated the PMA-induced oxidative burst of neutrophils. In both studies, the responses observed were attenuated in the presence of RU486. In conclusion, DEX may attenuate neutrophil inflammatory response through receptor-mediated genomic and non-genomic pathways. This finding may contribute to the rapid response (minutes) observed following corticosteroid administration in selected equine inflammatory processes.

Fast Food Consumption, Overweight, and Asthma in Preadolescents

Xiao-Mei Mai, Elizabeth A.C. Sellers, Allan B. Becker, Anita L. Kozyrskyj, Faculty of Pharmacy, Pediatrics and Child Health and Manitoba Institute of Child Health, University of Manitoba, Winnipeg, MB

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 to 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 ≥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 of 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 of 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.

Leptin Is Associated with Asthma in Overweight Children

Xiao-Mei Mai, Carla Taylor, Allan B. Becker, Anita L. Kozyrskyj, Faculty of Pharmacy, Pediatrics and Child Health and Manitoba Institute of Child Health, Human Nutritional Sciences, University of Manitoba, Winnipeg, MB

Background: Leptin is an inflammatory adipokine associated with obesity and cardiovascular disease. *Aims:* To evaluate the levels of serum leptin in relation to asthma in overweight and normal weight children. *Materials:* 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) ≥85th percentile of gender- and age-specific growth charts. The serum levels of leptin were analyzed by ELISA. *Results:* Overweight was associated with threefold increases in leptin compared with normal weight (geometric mean [GM] 13.87 vs 4.14 ng/mL, $p < .0001$). The levels of leptin were higher in asthmatic than non-asthmatic children (GM 7.32 vs 5.37 ng/mL, $p = .03$). Differences by asthma status were significant in overweight (GM 20.29 vs 10.49 ng/mL, $p < .001$) but not normal-weight children (GM 4.39 vs 3.94 ng/mL, $p = .45$). In overweight children, multiple linear regression analysis showed that leptin was increased 1.7 times in asthmatic versus non-asthmatic children ($p = .003$), independent of gender, age, BMI percentile, maternal and paternal asthma, current passive smoke, and breast-feeding. Besides asthma status, female gender and increase in BMI percentile predicted elevated levels of leptin in overweight children ($p < .001$ for both). *Conclusions:* Leptin is increased in children with asthma, particularly those who are overweight and may play a role in airway inflammation in those children.

Adjuvant Monoclonal Antibody Treatment in a Patient with Allergic Asthma and Previously Failed Immunotherapy

Kyla J. Hildebrand, Timothy Vander Leek, Per Lidman, Stuart Carr

A 6-year-old patient was referred with a 2-year history of persistent nasal congestion and intermittent wheeze, dyspnea, and chest tightness. Symptoms were exacerbated by cats and dust, with no seasonal component. Initial spirometry revealed FEV1/FVC of 75%, FEF 25 to 75% was 48% predicted, and there was a 19% increase in FEV1 post-bronchodilator. Skin testing was positive for cats and dogs but negative for other common aeroallergens. Diagnoses of allergic asthma and rhinoconjunctivitis were made and treatment was initiated with intranasal mometasone, inhaled HFA-beclomethasone 200 μg bid, and salbutamol as needed. Lung function remained inadequately controlled, and she was started on budesonide/formoterol 200/6 μg 2 puffs bid instead. Reversible airflow obstruction persisted, and her symptoms remained consistently worse with unavoidable or secondhand animal exposures. Therefore, she was considered a candidate for cat and dog allergen immunotherapy. She was unable to achieve maintenance immunotherapy, repeatedly developing anaphylaxis (expiratory wheeze, respiratory distress, and marked airflow obstruction on spirometry) and requiring epinephrine on several occasions. Omalizumab is a humanized monoclonal antibody directed against the Fc component of human IgE, preventing binding to the high-affinity IgE receptor (FceRI) on mast cells and basophils. It has been approved as adjuvant treatment for moderate-to-severe allergic asthma. Our patient started omalizumab 150 mg monthly, and specific immunotherapy was continued to modify her immune response in the longer term. She was able to tolerate increasing immunotherapy doses without incident and successfully achieved maintenance shortly after starting omalizumab. With a year of combined therapy, her asthma symptoms have improved significantly, and she has tolerated a reduction in her budesonide/formoterol dose. She also tolerates unavoidable animal exposures with only mild symptoms. There have been several small studies of omalizumab and immunotherapy for seasonal rhinitis. We believe this to be the first case report using omalizumab to facilitate previously failed immunotherapy in a patient with allergic asthma.

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

L. Yallop, P. McGrath, B. MacNeil, A.B. Becker, A.L. Kozyrskyj

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 2005-2006 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. Twenty percent of the mothers in the sample had postpartum depression as defined by the database measure. Forty-seven percent of mothers had some amount of postpartum depression according to self-report. Of the mothers who reported postpartum depression, 17% continued to experience depression 10 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 1 year following birth, is a valid method to

identify postpartum depression in population-based health care database studies.

Bacterial Infections Including Pericarditis in Two New Cases of Interleukin-1 Receptor-Activated Kinase 4 Deficiency: Importance of Early Imaging to Diagnose "Silent Infections" and Blood IL-6 Assay to Direct Definitive Diagnosis

Andrew C. Issekutz, Tong-Jun Lin, Marian B. Macken, Cheng-Lung Ku, Horst von Bernuth, Jean-Laurent Casanova, Departments of Pediatrics and Diagnostic Imaging, Dalhousie University, Halifax, NS; Laboratory of Human Genetics of Infectious Diseases, University of Paris René Descartes, Paris, France; Pediatric Immunology - Hematology Unit, Necker-Enfants Malades Medical School, Paris, France

We present two unrelated Canadian patients with interleukin-1 receptor-activated kinase 4 (IRAK-4) deficiency, a defect in innate immunity. Patient 1, a 16-year-old boy, had pneumococcal meningitis at 14 months, *S. aureus* pericarditis at 20 months, and then osteomyelitis, staphylococcal cellulitis, and at age 10 years multiple liver abscesses progressing silently to rupture and generalized peritonitis due to *S. aureus*. Patient 2 presented at 4 months with a left cervical mass, but a CT scan revealed extensive bilateral cervical suppurative adenitis due to *S. aureus*. After therapy and resolution, at 8 months a mass recurred in the right submandibular region and CT scans showed multiple lymph node abscesses, including in the paratracheal nodes, yielding *S. aureus*. Both patients have been well (#1 for 6 years, #2 for 12 months) on cotrimoxazole ± penicillin prophylaxis and repeated immunization with pneumococcal vaccine. Presentations were remarkable for minimal or no fever, leukocytosis, ESR, and CRP response to these infections. Symptoms were vague, nonspecific, and delayed. Ultrasound and CT scans revealed extensive involvement of tissue and abscess formation, often involving areas not clinically suspected. Stimulation of blood leukocytes with IL-1β, bacterial endotoxin (LPS), peptidoglycan, or poly-IC failed to induce IL-6 production (<200 pg/mL; normal donors 1,000-16,000 pg/mL depending on stimulus). The TNF-α response was similarly deficient. Both patients had IRAK-4 gene mutations. Thus, IRAK-4 deficiency can present with staphylococcal pericarditis. This immunodeficiency is important to consider when there are repeated or extensive bacterial infections but minimal symptoms and acute-phase response. Stimulated whole-blood IL-6 or TNF-α production is of great value for a presumptive diagnosis, prior to sequencing of IRAK-4 genes. *Support:* Canadian Institutes of Health Research (CIHR).

Peripheral Blood Leukocytes from Asthmatic Horses (Heaves) Present an Aberrant Response to Bacterial Extracts

A. Lavoie-Lamoureux, J. Martin, J.-P. Lavoie, Department of Clinical Sciences, Veterinary Medicine Faculty, University of Montreal, Montreal, QC; Department of Physiology, Meakins-Christie Laboratories, McGill University, Montreal, QC

Rationale: It has been suggested that susceptibility of allergic individual to LPS and other bacterial wall products found in house dust predisposes asthmatic individuals to the onset of clinical exacerbations. Here we compare the response to bacterial wall components of neutrophils and other peripheral blood (PB) leukocytes from horses with heaves with that of healthy controls. *Methods:* Neutrophils from control (n = 5) and heave-susceptible horses (n = 6), isolated from PB using an immunomagnetic technique, were stimulated for 5 hours in the presence of LPS (100 ηg/mL) and fMLP (10^{-8} M). Differential mRNA expression was studied using real-time PCR. In separated experiments, neutrophils were stimulated for 1 hour with LPS (100 ηg/mL or 1 μg/mL) to assess integrin expression (CD18) using flow cytometry. Concurrently, the neutrophil-depleted PB leukocyte fraction was cultured in the presence of LPS and fMLP for 5 hours to evaluate gene expression. *Results:* Compared with control animals, LPS- and fMLP-stimulated neutrophils from heave-susceptible horses had a significant decreased expression of IL-8 and an increased expression of TNF-α. Moreover, neutrophil-depleted PB leukocytes from heave-affected horses had a greater expression of proinflammatory 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 heave-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.

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, MB

Glucocorticoids are potent anti-inflammatory drugs that inhibit apoptosis of human neutrophils through

unknown mechanisms. In this study we show that dexamethasone, a classic 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 prosurvival protein Mcl-1 were associated with decreased translocation and cleavage of the proapoptotic 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 antiapoptotic response to glucocorticoids by enhancing prosurvival proteins and blocking the intrinsic pathway of apoptosis.

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

N. Saurek-Aleksandrovska, A.L. Kozyrskyj, R. Rabbani, A.B. Becker, E.A.C. Sellers

Rationale: To validate the ^{13}C 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 ^{13}C 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 ^{13}C 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). Fifteen percent had a significant degree of IR as measured by HOMA-IR, 6% as measured by the ^{13}C breath test. Using HOMA-IR as a standard, the sensitivity of the ^{13}C glucose breath test was 16% (0.07-0.27), and the specificity was 96% (0.93-0.98). The Pearson correlation between ^{13}C breath test and HOMA-IR was r = -.345, p < .0001. From the multiple regression results (r^2 = .278), significant predictors of HOMA-IR were the BMI z-score (B = 0.611, p < .0001) and the interaction between BMI z-score and ^{13}C values (B = -0.019, p < .0001). No significant associations were found between ^{13}C and asthma status, even following stratification for overweight status. *Conclusions:* The ^{13}C 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.

Exogenous Nitric Oxide Regulates Cyclooxygenase-2 Expression and Prostaglandin D_2 Generation in Mouse Bone Marrow-Derived Mast Cells

Tae Chul Moon, A. Dean Befus, Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB

Mast cells (MCs) 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 an 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) D_2 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 (BMMCs) exhibit biphasic PGD_2 biosynthesis; COX-1-dependent immediate and COX-2-dependent delayed PGD_2 production following COX-2 expression, when BMMCs are stimulated with stem cell factor (SCF), IL-10, and IL-1β. The role of NO on COX-2 expression and PGD_2 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 PGD_2 generation in response to SCF, IL-10, and IL-1β. Even though both SNAP and SNOG augmented COX-2 protein expression and COX-2-dependent PGD_2 generation, no effects of NO donors were observed on COX-2 mRNA expression after 2 hours 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 (100-500 μM) but not SNAP (up to 500 μM) inhibited COX-1-dependent PGD_2 generation. These results suggest that exogenous NO regulates PGD_2 production by MCs in inflammatory states through regulation both of COX-1 and COX-2. Furthermore, these findings help us understand the role of NO in MC function and the regulatory mechanisms of lipid mediator generation in MCs in inflammatory disease. *Support:* CIHR and the Korea Research Foundation (KRF-2005-214-C00111).

Endotoxin Protection against Atopy: Phenotypic Differences between First Nations and Non-First Nations Children

S. Huq, A.B. Becker, A.L. 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-FN 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 to 10 years. FN or Metis status was self-declared in 150 children, 55% living on a 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 hyperresponsiveness) 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 to 97 eu/g, 98 to 141 eu/g, and >141 eu/g, adjusted for gender, maternal asthma, mould and tobacco smoke exposure at birth, and current mould, was determined in logistic regression analyses. *Results:* Mean endotoxin levels were significantly higher in FN homes than in non-FN homes (189.9 vs 108.7 eu/g, p = .03). Endotoxin was protective against atopic dermatitis in non-FN children, with adjusted ORs of 0.47 (95% CI 0.23-0.97) for midquartile 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.

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

S. Huq, A.B. Becker, A.L. Kozyrskyj

Rationale: First Nations' (FN) homes have high levels of indoor mould. While indoor mould 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 mould in FN and non-FN 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 to 10 years. FN status was self-declared in 150 children, 55% living on a reserve. Information on current mould exposure was obtained from home inspection and a survey questionnaire. Bronchial hyperresponsiveness (BHR) 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 mould exposure, adjusted for gender, maternal asthma, mould and tobacco smoke exposure at birth, and endotoxin levels, was determined in logistic regression analyses. *Results:* Sixty-eight percent of FN and 67% of non-FN children had current mould in the home. Asthma was three 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 mould and asthma phenotypes in non-FN children. *Conclusions:* Indoor mould 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 mould may be acting as a respiratory irritant in FN homes. The extent of exposure to mould may be determined by the levels of β-glucan in dust samples, which are currently being assayed.

Comparison of Ciclesonide Uptake and Activation in Hypotonic Versus Isotonic Suspensions and Fatty Acid Conjugate Formation of Desisobutyryl-Ciclesonide in a Hypotonic Ciclesonide Suspension in Rabbit Nasal Mucosa

R. Nave, H. Sato, T. Nonaka, M. Wingertzahn, T. Mochizuki, S. Takahama, S. Kondo, ALTANA Pharma AG, Konstanz, Germany; Teijin Institute for Biomedical Research, Teijin Pharma Limited, Tokyo, Japan; ALTANA Pharma, Florham Park, NJ

Background: Ciclesonide (CIC) is a novel corticosteroid under development for treatment of asthma and allergic rhinitis (AR). CIC is converted to pharmacologically active desisobytyryl-ciclesonide (des-CIC) by upper and lower airway intracellular esterases. Unlike available isotonic intranasal corticosteroid suspensions, CIC is formulated in a hypotonic suspension that may enhance delivery of CIC to nasal mucosa. The purpose of this study was to evaluate the in vivo uptake and activation of CIC to des-CIC in hypotonic and isotonic suspensions and confirm the formation of fatty acid conjugates of des-CIC when delivered to the nasal mucosa of rabbits as a hypotonic suspension. *Method:* The uptake of CIC and its activation were compared in a hypotonic suspension versus an isotonic suspension in nasal

mucosa of Japanese white rabbits. Both suspensions were administered intranasally as a single dose (143 μg/animal, 4 animals/suspension). Ciclesonide and des-CIC concentrations were measured in nasal mucosa extracts (0.5, 2, and 4 hours post-administration) using high-performance liquid chromatography with tandem mass spectrometric detection. Subsequently, retention of des-CIC and formation of fatty acid conjugates of des-CIC (des-CIC-oleate and des-CIC-palmitate) were determined in nasal mucosa extracts (5 animals/time point) 0.5, 8, 12, 16, and 24 hours post-administration of a hypotonic CIC suspension. *Results:* The hypotonic suspension achieved higher concentrations of CIC (25.3-fold, 34.2-fold [p = NS], and 16-fold [$p < .05$]) and des-CIC (5.7-fold, 11.6-fold, and 13.7-fold; $p < .05$ for all) at 0.5, 2, and 4 hours post-administration, respectively, versus isotonic suspension. Furthermore, it was determined that des-CIC is further metabolized to form inactive fatty acid conjugates. The highest mean concentrations of des-CIC-oleate (167.5 pmol/g tissue) and des-CIC-palmitate (3.1 pmol/g tissue) were present in nasal mucosa at 8 hours and 16 hours, respectively. des-CIC, des-CIC-oleate, and des-CIC-palmitate were present up to 24 hours post-administration (45.5, 50.3, and 1.3 pmol/g tissue, respectively). *Conclusion:* A hypotonic CIC suspension provides better absorption of CIC in vivo and higher tissue concentrations of des-CIC compared with an isotonic suspension. Similar to findings in the lung, the formation of reversible fatty acid conjugates of des-CIC, which may serve as a slow-release pool of des-CIC, was confirmed in rabbit nasal tissue.

Airway-Specific Activation and Metabolism of the Corticosteroid Ciclesonide

R. Nave, H. Sato, T. Nonaka, T. Mochizuki, S. Takahama, S. Kondo, Altana Pharma AG, Konstanz, Germany; Teijin Institute for Bio-Medical Research, Teijin Pharma Limited, Tokyo, Japan

Rationale: Ciclesonide (CIC) is a novel corticosteroid in clinical development for the treatment of asthma and allergic rhinitis. Previous studies have shown that CIC is converted to a pharmacologically active metabolite, desisobutyryl-ciclesonide (des-CIC), by endogenous esterases in human lung. However, CIC metabolism in the nose has not been well established. Therefore, a series of studies have been conducted to characterize CIC metabolism in nasal mucosa. *Methods:* Human nasal epithelial cells (HNECs) were incubated with CIC 0.1 μM for 1 hour, and metabolite concentrations were measured over 24 hours. The reversibility of fatty acid conjugates of des-CIC was assessed by incubating HNEC with des-CIC 1 μM for 6 hours and then monitoring intra- and extracellular metabolite concentrations over 24 hours. Specific esterases that convert CIC to des-CIC were identified by preincubating HNECs with esterase inhibitors for 30 minutes prior to incubation with CIC 5 μM for 6 hours. Compound concentrations were measured using high-performance liquid chromatography with tandem mass spectrometry. *Results:* CIC metabolites were detected at all time points after incubation of HNECs with CIC. Intracellular des-CIC and fatty acid conjugates of des-CIC were still detectable 24 hours after removal of des-CIC from the medium. In the absence of esterase inhibitors, ≅90% of CIC was metabolized to des-CIC and fatty acid conjugates of des-CIC. Carboxylesterase and cholinesterase inhibitors caused a dose-dependent decrease in CIC metabolism. *Conclusions:* These data confirm CIC activation to des-CIC in nasal mucosa via a similar mechanism to that observed in the lung. Ciclesonide is rapidly converted to des-CIC by carboxylesterases and cholinesterases, and reversible fatty acid conjugates of des-CIC are maintained intracellularly for at least 24 hours.

Efficacy and Safety of Ciclesonide for the Treatment of Perennial Allergic Rhinitis

E.O. Meltzer, S. Kunjibettu, N. Hall, W. Berger, C. LaForce, P.F. Darken, T. Shah, M.A. Wingertzahn, Allergy and Asthma Medical Group and Research Center, San Diego, CA; Altana Pharma, Florham Park, NJ; Allergy and Asthma Associates of Southern California, Mission Viejo, CA; North Carolina Clinical Research, Raleigh, NC

Rationale: Ciclesonide is a new corticosteroid under investigation for the treatment of allergic rhinitis. Study objectives were to demonstrate the efficacy of intranasal CIC 200 μg once daily (OD) in the treatment of PAR and to assess quality-of-life effects and safety. *Methods:* In a phase III, multicenter, randomized, double-blind, placebo-controlled study, adults and adolescents (n = 471) with a ≥2-year history of PAR received intranasal administration of CIC 200 μg (2 sprays per nostril) or placebo OD for 6 weeks. Patient-assessed total nasal symptom score (TNSS), physician-assessed overall nasal signs and symptoms severity (PANS), and quality of life as assessed by the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) were evaluated. *Results:* Ciclesonide significantly reduced the average am and pm reflective TNSS compared with placebo after 6 weeks of treatment ($p < .001$). Additionally, ciclesonide significantly reduced the average am and pm instantaneous TNSS after 6 weeks of treatment (p = .001). At end point, a greater decrease from baseline was observed in the PANS for the CIC group (p = .051 versus placebo). An appreciable improvement in the combined RQLQ scores at end point was also observed in patients treated with CIC compared with placebo (p = .011). The frequency of adverse events was similar among patients treated with CIC or placebo. *Conclusions:* Ciclesonide administered intranasally was

significantly superior to placebo in relieving nasal symptoms and provided appreciable improvement in health-related quality of life in adult and adolescent patients with PAR. Ciclesonide was also well tolerated with a safety profile that was comparable with placebo.

Efficacy and Safety of Ciclesonide Nasal Spray for the Treatment of Seasonal Allergic Rhinitis

P.H. Ratner, J.H. van Bavel, F. Hampel, P.F. Darken, N. Hall, T. Shah, M.A. Wingertzahn, Sylvana Research, San Antonio, TX; Allergy and Asthma Associates, PLLC, Austin, TX; Central Texas Health Research, New Braunfels, TX; Altana Pharma, Florham Park, NJ

Rationale: Intranasal corticosteroids are considered the most effective treatment for the management of allergic rhinitis (AR) symptoms. The objective of this study was to evaluate the efficacy and safety of a new corticosteroid in development for the treatment of asthma and AR, ciclesonide (CIC), in patients with seasonal AR (SAR). *Methods:* In this phase III, double-blind, placebo-controlled trial, patients with SAR (age ≥ 12 years) were randomized to receive intranasal CIC 200 μg (n = 164) or placebo (n = 163) once daily for 28 days. Patient-assessed total nasal symptom score (TNSS), physician-assessed overall nasal signs and symptoms severity (PANS), and Rhinoconjunctivitis Quality-of-Life Questionnaire (RQLQ) were evaluated. Adverse events (AEs) were monitored throughout the study. *Results:* CIC significantly improved the average of am and pm reflective TNSS and instantaneous TNSS versus placebo from days 1 through 14 (primary end point) and over the 28-day treatment period (p < .001). Furthermore, CIC showed similar improvements in both am and pm placebo-adjusted reflective TNSS, suggesting a 24-hour effect. At day 15, CIC improved RQLQ by -1.17 ± 0.10 compared with improvement of -0.72 ± 0.10 with placebo (p = .002). The frequency of treatment-related AEs was low and comparable between treatment groups, and the placebo-adjusted epistaxis rate for CIC was 1.8%. *Conclusions:* CIC administered intranasally was superior to placebo in relieving nasal symptoms in adult and adolescent patients with SAR. CIC was well tolerated, with a safety profile comparable with placebo and a low epistaxis rate. These data support the continued clinical development of CIC nasal spray.

Interleukin-17 Enhances Interleukin-1β-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, Departments of Immunology and Physiology and Sections of Respiratory Diseases and Thoracic Surgery, University of Manitoba, Winnipeg, MB

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-1β-mediated CXCL-8 release from human airway smooth muscle cells (HASMCs) 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 HASMC cultures. HASMCs transfected with site-mutated AP-1/NFκB 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-κB promoter binding sites. We demonstrate IL-17 induced and synergized with IL-1β to up-regulate CXCL-8 mRNA and protein levels. Erk-1/2 and p38 modulated IL-17 and IL-1β CXCL-8 promoter activity; however, IL-1β 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-κB 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-1β to enhance CXCL-8 promoter activity, mRNA induction, and protein synthesis in HASMCs via the cooperative activation of AP-1 and NF-κB trans-acting elements.

Immunologic Responses in a Murine Model Following Exposure to Beryllium Metal Particles

Fariba Salehi, Séverine Audusseau, Natalya Karp, Joseph Zayed, Bruce Mazer, Meakins-Christie Laboratories, McGill University, Montreal, QC; University of Montreal, Montreal, QC

Chronic beryllium disease (CBD) is a progressive granulomatous lung disease. CBD is caused by exposure to beryllium (Be) and is characterized by mononuclear cell infiltration and granulomatous inflammation in the lung. A proposed immunologic basis for this disease states that affected individuals have a Be-specific hypersensitivity response involving $CD4^+$ T lymphocytes. The objective of this study is to determine the immunologic effects of different particles size of Be metal on C3H/HeJ mice following a nose-only inhalation exposure. We examined the role of Be metal, total and respirable particles, in the development of granulomatous inflammation in the lung, proliferation of T lymphocytes, and production of Th1-type cytokines. The beryllium lymphocyte proliferation test was used to explore the presence of a beryllium-specific immune response in the lung and spleen. Although our results did not show any significant difference or higher stimulation index (SI) among groups, the proliferation was higher for exposed

groups than control (620 CPM vs 350 CPM). The standard histology of the lung showed granulomatous inflammation that may be more evident by extending the exposure period. Flow-cytometric analysis of cells stained with fluorescence labeled antibodies for intracellular cytokine and surface markers indicated a significantly greater expression of $CD4^+$, $CD8^+$, and IFN-γ for both Be particles. Cytokine assays of bronchoalveolar lavage revealed higher amounts of IL-12 and IFN-γ in Be-exposed groups. Taken together, this study is a unique murine model to explore the immunologic effect of Be particles to induce lung disease that may enhance our knowledge to identify a scientifically based threshold to protect workers against CBD.

Eosinophil-Derived Tryptophan Catabolites Inhibit Lymphocyte Survival via Glutamate Receptors

M. Ghaffari, S.O. Odemuyiwa, V. Lam, A. Ghahary, M. Duszyk, R. Moqbel, Departments of Medicine and Physiology, Pulmonary Research Group, University of Alberta, Edmonton, AB; Department of Plastic Surgery, University of British Columbia, Vancouver, BC

Aim of Study: Eosinophils express indoleamine 2,3-dioxygenase, which catalyzes 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 an 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 a 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 up-regulated following activation with phytohemagglutinin. 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 100 pg/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.

Allergen-Specific T and B Cells in Allergic Patients Are Not Increased Compared with Nonallergic Persons

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

Background: Allergic disease is thought to be caused by increased (compared with nonallergic state) production by plasma cells of IgE against innocuous substances such as 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 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 nonallergic 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 Th2 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 nonallergic controls. *Conclusion:* This suggests that allergic and nonallergic B cells differ not in the number but in their propensity to differentiate into plasma cells producing large amounts of IgE.

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

Yokananth Sekar, A. Dean Befus, Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB

Mast cells (MCs) are effector cells of IgE-mediated allergic diseases. MCs 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 antinitrotyrosine antibody. Mass spectrometry was used to characterize proteins selectively nitrated upon treating the cells with SNOG, a NO donor. A 500 μM of SNOG for 4 hours selectively nitrated aldolase A in HMC-1 cells. Western blot analysis with antialdolase antibody revealed that there are multiple isoforms of aldolases with the 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 the 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 MCs, 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 on 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 on its nitration may play a regulatory role on MC phenotype and function. *Acknowledgement:* The Canadian Institutes of Health Research and Alberta Lung Association.

Respiratory Syncytial Virus-Infected Dendritic Cells Induce Specific CD3CD4CD25 Lymphocytes and Eosinophil Activation

Francis Davoine, Redwan Moqbel, Darryl Adamko, Campus Saint-Jean and Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB

Rationale: Asthma exacerbation is among the principal causes of pediatric visits to emergency clinics. Respiratory syncytial virus (RSV) infection in early childhood is often associated with inflammatory asthma development. Benign viral respiratory tract infections in the days or weeks prior to asthma exacerbation are also frequently reported by patients and parents. Dendritic cells (DCs) and T lymphocytes play an important role in the regulation of the immune response while eosinophils play a key role in the inflammation observed in asthma. *Hypothesis:* Virus induces DC and T cell priming that leads to eosinophils activation. *Methods:* Primary DC cultures were infected with RSV and cocultured with purified T cells. Proliferation and phenotyping of DCs (CD11c and HLA-DR) and T cells (CD4, CD8, CD3, and CD25) were done using four-colour flow cytometry. Measurement of eosinophils activation was done using OPD colorimetric assay to determine eosinophil peroxidase (EPO) release. *Results:* We observed an increased expression of CD11c and HLA-DR on DCs induced by RSV. Coculture of RSV, DCs, and T cells induced a specific increase of CD3CD4CD25 subpopulation (16 ± 3%, $n = 4$, $p < .05$) compared with uninfected coculture controls (6 ± 2%). Media of RSV, DCs, and T cells cocultures induced release of EPO by eosinophils. No effect was observed in single-population conditions. *Conclusion:* RSV is capable of inducing activation of a specific subset of T cells only when cocultured with activated DCs. Therefore, this activation induces the release of factors that lead to eosinophils activation and cytotoxic granule release. The mechanism of RSV-DCs-T cells interactions is not yet resolved, but it might provide a new understanding of the relationship between viral respiratory tract infections and exacerbation of asthma.

Respiratory Syncytial Virus Infection Influences the Immune System toward a Th2 Response: The Role of Indoleamine 2,3-Dioxygenase

Farnam Ajamian, Francis Davoine, Solomon O. Odemuyiwa, Redwan Moqbel, Darryl Adamko, Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB

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 the 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 polarization 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 DCs 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 μM) compared with uninfected control DCs (10 μM) (n = 2). *Conclusion:* RSV infects THP1 and DCs in vitro. DC 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.

Airway Side Population Cells: Progenitors of Multiple Cell Types

T.-L. Hackett, F. Shaheen, G. Singhera, D. Dorscheid, S. Van Eeden, R. Hegele, D.A. Knight, 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 characterize 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 was composed 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 characterize these cells.

Lower Cortisol Levels Associated with Childhood Asthma: The Role of Maternal Depression

L. Yallop, B. MacNeil, A.B. Becker, A.L. Kozyrskyj

Rationale: Maternal depression has been associated with abnormal cortisol levels and in other studies with asthma in children. We investigated the relationship between cortisol levels, maternal depression, and asthma in school-age children. *Methods:* Data were obtained from the SAGE case-control study of the 1995 Manitoba birth cohort. Mothers were surveyed on the presence of postpartum and current depression (PHQ questionnaire). Thirty-six mothers were found to have recurrent depression. Asthma in the child was diagnosed by a pediatric allergist and blood samples were assayed for cortisol levels using ELISA. Logistic regression was conducted with *SPSS* to predict the association between cortisol levels and asthma (odds ratio, OR), adjusted for treatment with inhaled corticoteroids (ICS) and frequency of health care. *Results:* Morning plasma cortisol levels measured in 595 children, ages 8 to 10 years, ranged from 3.1 to 254.8 ng/mL (mean 50.3 ng/mL). Children with asthma had lower mean cortisol levels compared with non-asthmatic children (47.2 ng/mL vs 52.1 ng/mL; p = .05). Mean cortisol levels were lower (46.6 ng/mL) in children of mothers with recurrent depression than those without, but not significantly so. Normal morning cortisol levels (>50 ng/mL) were 30% less likely in children with asthma (unadjusted OR 0.71, 95% CI 0.49-1.01). Compared with children with normal cortisol levels/not receiving ICS, the OR for asthma in children with normal cortisol levels/ICS use was 36.9 (95% CI 7.83-173), with low cortisol levels/ICS use was 10.4 (95% CI 4.56-23.7), and with low cortisol levels/no ICS use was 1.82 (1.03-3.24). Maternal recurrent depression increased the association between low cortisol levels and asthma among children not receiving ICS. *Conclusions:* Not surprisingly, ICS users were mostly likely to be asthmatic children, and ICS lowered cortisol levels in some children. However, in the absence of ICS use, low cortisol levels were more likely in children with asthma, and this association was modified by maternal recurrent depression.

Age-Dependent Ontogeny of Toll-Like Receptor 4 Expression on Peripheral Lymphocytes and Their Suppression by a T Helper 2 Inflammatory Cytokine

Jella An, Andrée-Anne Banville-Langelier, Pierre Fiset, Julie Guay, Oumnia Hajoui, Qutayba Hamid, Bruce Mazer, Meakins-Christie Laboratories, McGill University, Montreal, QC

The hygiene hypothesis suggests that decreased exposure to pathogenic microbes in early life is a potential cause of the rapid increase of allergic diseases. Microbial products such as lipopolysaccharide (LPS) have been

shown to antagonize T helper type 2 (Th2) responses implicated in allergic disease upon binding Toll-like receptor 4 (TLR4), but this protective effect was only seen in children. Recent studies have shown that $CD4^+$ natural killer (NK) T cells may play a prominent role in the pathogenesis of allergic diseases via rapid production of Th2 cytokines. To determine the expression and function of TLR4 on peripheral lymphocytes and its dependency on age and atopic status, we used three-colour flow cytometry to analyze stained cells for atopic and nonatopic children and adults (n = 120). We also studied the effect of interleukin 4 (IL-4) and LPS stimulation on TLR4 immunopositive cells, simulating ongoing allergic inflammation and microbial infection, respectively. We found that TLR4 expression is significantly higher on peripheral T (7.72 ± 2.1% vs 2.21 ± 0.7%) and B cells (38.99 ± 4.4% vs 20.45 ± 6.3%) and lower on NK T cells (4.09 ± 1.0% vs 8.31 ± 2.9%) in children compared with adults, regardless of atopic status. In each patient group, IL-4 down-regulated $TLR4^+CD4^+$ T cells but up-regulated $TLR4^+CD4^+$ NK T cells, whereas it had little effect on $TLR4^+$B cells. As well, IL-4 increased IL-4 production by NKT cells, detected by intracellular staining. LPS did not affect TLR4 expression on peripheral lymphocytes or NK T-cell cytokine production after 24 hours of stimulation. In conclusion, our results confirm that the expression and function of TLR4 on peripheral lymphocytes are dependent on age, potentially explaining the increased susceptibility to immune regulation by LPS in children. The observed suppression of $TLR4^+$ T cells and activation of NK T cells by the Th2-type inflammatory cytokine may also explain the chronicity of allergic disease.

Allergy Management Behaviours and Sources of Stress for Schools and Families Living with Life-Threatening Food Allergies

Janice Butler, Newfoundland and Labrador Centre for Applied Health Research, Faculty of Medicine, Memorial University, St. John's, NF

The purpose of this study was to describe the approaches taken by individuals, families, and school staff to the management of life-threatening food allergies and to describe the sources of stress associated with managing this condition. This was a cross-sectional descriptive study employing both quantitative and qualitative research methods using two instruments developed for the study. In 2003, the principals from 40 randomly selected schools in Newfoundland and Labrador were interviewed, as were 25 parents of children with food allergies attending schools in the province; 21 of the parents and 4 teens each also participated in one of four focus groups. The results suggested that the reported number of students with food allergies in provincial schools has increased. While most schools demonstrated a positive approach to managing students with food allergies, one-quarter of the schools studied had many deficiencies in allergy management that may contribute to negative outcomes for families living with this stressful condition. Balancing individual rights, feelings of uncertainty, and increased workload were sources of stress for principals. Many parents of children with life-threatening food allergies reportedly felt inadequately prepared by health professionals to safely manage their child's food allergy. Parents also identified inconsistent allergy management among schools in this province. The sources of stress for families included a perceived lack of control over allergies, inadequate public understanding and support, and the lack of reprieve from daily worries. The results of this study support the need for a comprehensive provincial school policy on food allergy management, improved education by health professionals for school staff and families living with food allergies, and a public education and awareness campaign to increase understanding about the challenges of living with food allergies.

Asthma Economics Conference

Mark Greenwald, Noel Kerin, Frank P. Fiti, Asthma Society of Canada, Toronto, ON

Asthma, as one of the most prevalent chronic diseases in Canada, generates a high burden in both private industry and society at large. In June 2005, the Asthma Society of Canada and the Schulich School of Business brought together expertise in health care, public policy, and economics to integrate diverse viewpoints and ideas about asthma management in Canada. Through interactive workshops, participants identified major strengths and weaknesses of current asthma care in Canada. A common theme of the conference was the inability of traditional measures of disease burden to adequately assess the total burden of chronic diseases, such as asthma. Traditional mechanisms measure direct costs of the disease in the form of increased direct spending on the health care system. However, indirect costs, in the form of lost time at work, presenteeism (lost productivity of workers who attend work but are unable to maintain productivity due to illness), and missed school days, are not measured. Likewise, the effects on the economy of lower productivity are not measured. Present estimates of indirect costs are not tracked and are believed by some to approach the direct costs to the health care system. Changing the attitude of policy makers from a "preventing costs" mindset to an "investment for future returns" mindset will allow for the recognition that investing the necessary time and money in the health of workers and children now will yield substantially greater financial return in the years to come. The conference

participants suggested a number of changes and innovations to improve asthma care. They identified the need for a national and cohesive strategy for asthma management in Canada. A strategy that would focus on early identification of the disease, patient and health care provider education, and tracking the long- term costs of the disease and measuring the indirect costs noted above.

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

Nivedita Khannna, Pascal Djiadeu, Stacey A. Ritz, Nades Palaniyar, Frances Silverman, Jeremy A. Scott, Divisions of Occupational and Respiratory Medicine, Department of Medicine, University of Toronto, Toronto, ON; The Hospital for Sick Children Research Institute, Toronto, ON; Northern Ontario School of Medicine, Sudbury, ON; Gage Occupational and 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 hyperresponsiveness 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 days 0 and 7, followed by aerosol challenge (6% OVA in PBS or PBS alone) on days 14 to 16 (25 min/day). Twenty-four hours after the last exposure to the allergen, conscious, freely moving animals were exposed to CAP (200-1,000 $\mu g/m^3$) using the Harvard Ambient Particle Concentrator and ozone (200 ppb-2 ppm) for 4 hours. Pulmonary function and airway responsiveness were subsequently assessed using a ventilator-based system (flexiVent). Bronchoalveolar lavage was performed for further assessment of immune end points with Western blotting and ELISA (ie, cytokine profiles, surfactant protein [SP] A, and SP-D). *Results:* We observed increased maximum responsiveness (p = .007) and a trend toward decreased EC_{50} 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.

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

D. Préfontaine, P.O. Fiset, A.-A. Banville-Langelier, M. Mazer, Q. Hamid, B. Mazer, Meakins-Christie Laboratories, McGill University, Montreal, QC

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 down-regulates 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 nonatopic controls or unstimulated cells and that Th2 transcription factors will correlate with TLR4 down-regulation. We hypothesized that atopy and Th2 conditions inhibit T-reg cell phenotype and function. *Methods:* Peripheral blood mononuclear cells (PBMCs) isolated from atopic and nonatopic children (2-18 years old) were incubated for 24 hours with or without IL-4 or LPS. RNA extracted from $CD4^{\pm}$ 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 PBMCs incubated with medium, IL-4, or LPS. *Results:* IL-4 stimulation of PBMCs 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 up-regulated FOXP3 gene expression in $CD4^+$ cells regardless of atopic status and increased its expression among $TLR4^+$ PBMCs. LPS also up-regulated both FOXP3 and IL-10 regulatory cytokine mRNA levels, whereas IL-4 down-regulated 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 down-regulation of IL-10.

Small Interfering Ribonucleic Acid Decreases Interleukin-13 Receptor α1 (IL-13Rα1) Expression and Signaling Through IL-13Rα1 in Human Tonsillar B Cells

Oumnia Hajoui, Julie Guay, Andrée-Anne Banville-Langelier, Bruce Mazer, Meakins Christie Laboratories, McGill University and McGill University Health Centre-Montreal Children's Hospital, Montreal, QC

Background: Our laboratory has demonstrated that human B lymphocytes synthesize IL-13 and that

production of this cytokine is crucial for maintaining IgE production. IL-13 mediates its effect through a complex receptor system, including IL-4Rα and IL-13Rα1. *Objective:* We evaluated if human IL-13Rα1 expression could be diminished by small interfering RNA (siRNA). *Methods:* Purified B cells were isolated from human tonsils. Three micrograms of siRNA for IL-13Rα1 (Dharmacon, Denver, CO) were transfected using the Human B Cell Nucleofector Kit (Amaxa Inc, USA) and then cultured. Expression of IL-13Rα1 mRNA was assessed by real-time PCR. Signal transduction via IL-13Rα1 was assessed by examination of phospho-STAT6$^+$ cells. Analysis of CD38 and CD27 expression on phospho-STAT6$^+$ cells was examined by flow cytometry before or after transfection with siRNA. *Results:* Transfection of B cells with IL-13Rα1-specific siRNA inhibited expression of IL-13Rα1 mRNA following 24 hours of anti-CD40/IL4 stimulation by 52%. Using the ratio of IL-13Rα1 mRNA/housekeeping-gene, untransfected B cells had a ratio of 4.65 ± 0.2 compared with 2.2 ± 1.64 for siRNA transfected cells. Control siRNA (Vector pmax-GFP) had no significant effect on IL-13Rα1 mRNA expression (ratio 4.11 ± 1.3). We examined signaling through IL-13Rα1 by examining increases in phospho-STAT6 by flow cytometry following addition of IL-13. IL-13 induced phospho-STAT6 expression in 69.3 ± 0.9% B cells; incubation with siRNA diminished this to 57.4 ± 0.4%. This was highly specific as there was no change in response to IL-4 following transfection with IL-13Rα1 siRNA. IL-13Rα1 was expressed on all B-cell lineages examined, and siRNA transfection diminished IL-13R signaling in both mature (CD38$^+$) and memory (CD27$^+$) B cells. *Conclusion:* Our results show that siRNA significantly inhibits IL-13Rα1 mRNA expression, but the inhibition using our current method of transfection is not complete. This is demonstrated by the small but significant inhibition of IL-13-mediated signal transduction. siRNA-mediated inhibition of IL-13Rα1 equally affects mature and memory B-cell subsets.

Identifying the Presence and Function of the FceR1 Receptor in Human Normal and Small Airway Epithelium

Elana Hochstadter, Julie Guay, Abdel Soussi-Gouni, Barbara Tolloczko, Bruce D. Mazer, Meakins Christie Laboratories, McGill University and McGill University Health Centre-Montreal Children's Hospital, Montreal, QC

Background: The FceR1 receptor is commonly found on mast cells and basophils and is known to bind to immunoglobulin IgE (IgE) with high affinity. Upon stimulation by an antigen, FceR1 transduces signals causing mast cell degranulation, resulting in the symptoms of hypersensitivity reactions such as allergic rhinitis, asthma, and systemic anaphylaxis. Recently, FceR1 has been found on tissues such as smooth muscle, which may contribute to airway reactivity. *Objective:* To determine the presence and function of the FceR1 receptor on normal airway epithelium (NAEC) and small airway epithelium (SAEC). *Methods:* Human normal and small airway epithelial cells lines were purchased from CLONETECH (Cambrex Corporation, East Rutherford, NJ) and the levels of receptor expression were determined using PCR and flow cytometry. To assess signal transduction, we measured intracellular Ca^{+2} ($[Ca^{2+}]i$) changes using fluorescent microscopy. FceR1 function was demonstrated by assessing cytokine mRNA following anti-IgE stimulation using conventional RT-PCR. *Results:* FceR1 mRNA was detected on both NAEC and SAEC by conventional RT-PCR. Flow cytometry demonstrated the expression of the FceR1 on both normal and small airway epithelium; however, there was greater expression of FceR1 on SAEC. Cross-linking of FceR1 on NAEC with anti-IgE lead to increases in $[Ca^{+2}]_i$. The mean baseline level of $[Ca^{2+}]i$ was approximately 40 nM, while the mean peak $[Ca^{2+}]i$ level was 160 nM. Real-time PCR demonstrated increased levels of mRNA for the cytokine IL-13 in NAEC and SAEC, with higher levels of mRNA found in the latter. *Conclusion:* Human airway epithelium expresses FceR1; however, there is a greater concentration of FceR1 on small airway versus normal airway epithelium. Cross-linking of FceR1 causes an increase in cytokine mRNA levels as well as intracellular calcium levels, demonstrating its involvement in cell activation. Signaling via FceR1 on airway epithelium may play an important role in inducing the inflammation found in allergic asthma.

Toll-Like Receptor 4 Expression on B Lymphocytes in Pediatric and Adult Populations

Andrée-Anne Banville-Langelier, Jella An, Julie Guay, Pierre Fiset, David Prefontaine, Monty Mazer, Qutayba Hamid, Bruce Mazer, Meakins Christie Laboratories, McGill University, and McGill University Health Centre-Montreal Children's Hospital, Montreal, QC

Background: Toll-like receptors (TLRs) are an integral part of innate immunity and allow cells to recognize and rapidly respond to important microbial patterns. Stimulation of TLR-4 by endotoxins in early life is associated with a lower incidence of allergic diseases; this effect appears to be lost in adulthood. Considerable focus has been put on the expression of TLR-4 on T cells and dendritic cells, but little is known about the expression and function of TLR-4 on B lymphocytes, which are responsible for the production of IgE. *Objectives:* We evaluated TLR-4 expression on peripheral B lymphocytes as part of a cross-sectional study in atopic and nonatopic children from ages 2 to 18. *Methods:* Subjects were recruited from the Pediatric Test Center

of the Montreal Children's Hospital and a questionnaire was administered to determine presence of a history of atopic diseases. Three to 5 cc of anticoagulated blood was taken to be stained for the presence of TLR-4 on all peripheral cell subsets. Aliquots were also incubated with interleukin-4 13.5 ng/mL or LPS 25 μg/mL for 24 hours prior to analysis. The cells were stained for presence of the pan-B lymphocyte marker CD19, TLR-4, and the presence of sIgD, CD38 and CD27. Data on other cell populations studied will be presented in accompanying abstracts. *Results:* Children ages 2 to 18 had an average of 39.3 ± 4% TLR^+ cell in their $CD19^+$ population, (n = 56) compared with adults (20 ± 6%) (n = 10). There was no significant difference between atopic and nonatopic children. TLR-4^+ B lymphocytes could be found on B cells of varying maturities: $CD19^+IgD^+$ (18 ± 4%) $CD19^+CD38^+$ (8 ± 0.6%) and memory B cells $CD19^+CD27^+$ (9 ± 2%). IL-4 did not cause any changes in B-cell TLR4 expression; however, incubation with LPS increased TLR4 expression on immature and mature B cells but not on memory B lymphocytes. *Conclusion:* B lymphocytes in children express TLR-4 on twice as many B lymphocytes as in comparable adult volunteers, and B cells in children exhibited signaling through TLR-4. LPS recognition may influence the production of IgE in pediatric atopic diseases.

Role of L-Arginine Metabolism in Murine Allergic Asthma

Michelle L. North, Nivedita Khanna, Hartmut Grasemann, Frances Silverman, Jeremy A. Scott, Divisions of Occupational and Respiratory Medicine, Department of Medicine, University of Toronto, The Hospital for Sick Children, and Gage Occupational and 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 use 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 to 20. Pulmonary function testing and methacholine responsiveness were 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 (ie, arginases 1 and 2, nNOS, iNOS, eNOS, and the cationic amino acid transporters), and arginase activity was determined in lung homogenates. *Results:* OVA-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 (ie, cationic amino acid transporters). *Conclusions:* These results support the contention 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. *Support:* AllerGen NCE, Ontario Thoracic Society/Glaxo Smith-Kline Award, National Sanitarium Association.

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, AB

Matrix metalloproteinase 9 (MMP-9) is released by lung epithelial cells (LECs) in conditions such as asthma and COPD. Expression of MMP-9 correlates with the 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. Phylogenic 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-γ changed WT1 expression. These treatments, however, induced WT1 translocation from the nucleus to the cytosol. Translocation was blocked with the NO synthase inhibitor 1400 W that also reduced MMP-9 gene expression and enzyme activity. WT1 knock-down through small interfering RNA (siRNA) up-regulated MMP-9 activity in the presence of 1400 W. 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.

Article Categories:

- Annual Scientific Meeting

Allergy, Asthma, and Clinical Immunology
Volume 02, Issue 04, December 2006, Pages 164-165
Annual Scientific Meeting

Addendum

Abstracts From The Annual Scientific Meeting, Winnipeg, September 22-25, 2005 Novel RAG1 mutation in a case of severe combined immunodeficiency

Adelle Atkinson, Junyan Zhang, Eyal Grunebaum and Chaim M. Roifman, Department of Paediatrics, The University of Toronto

Objective: Rearrangement of the V(D)J in B and T cells, which is essential to the development of normal immune function, depends on the recombinase activating enzymes RAG1 and RAG2. Mutations in RAG1 or RAG2 typically lead to a spectrum of disorders ranging from the B^-T^- SCID to Omenn's syndrome (OS). We present here a unique presentation of RAG1 deficiency. *Patient*: A 6 month-old girl presented with severe respiratory distress which deteriorated significantly in spite of antibiotic therapy. Eventually, there was some response to treatment with corticosteroids. She had normal number of circulating lymphocytes, no eosinophilia, erythroderma or lymphoid organ enlargement. *Results*: Investigation of the immune system done early on showed normal numbers of $CD3^+$ T cells which expressed either CD4 or CD8. Subsequent analysis of the T cell receptor repertoire demonstrated that nearly all $CD3^+$ T cells were clonal in origin. One clone expressed CD4 while the other expressed CD8. This extremely restricted T cell repertoire as well as the lack of circulating B cells prompted the analysis of RAG1 gene. This analysis revealed a novel homozygous T to C substitution at nucleotide position 2686 and confirmed the need for hematopoietic stem cell transplant. *Conclusions:* This case underscores that a high index of suspicion for the presence of an immunodeficiency and an in-depth analysis of the immune system is required even when the widely available flow cytometry standard analysis shows normal T cell numbers which express CD4 or CD8. Especially in the absence of circulating B cells. This more extensive analysis will aid in making the correct diagnosis such that appropriate management options can be considered.

Myeloperoxidase localization and release from the nucleus of neutrophils

Salahaddin Mahmudi-Azer, Furquan Shaheen, Andrew Sandford, Peter D. Paré, The James Hogg iCAPTURE Centre, St. Paul's Hospital, Dept. of Medicine, University of British Columbia, Canada.*

Myeloperoxidase (MPO) plays a central role in the pathogenesis of diverse infectious and inflammatory diseases. MPO has been shown to localize to neutrophil azurophilic granules and is released during cell activation and phagocytosis. We used a combination of immunofluorescent staining with confocal microscopy, immunogold-labelling and peroxidase staining with electron microscopy, flowcytometry of cell nuclei and subcellular fractions, electro-permeabilization with MPO secretion assays, a leptomycin-B (LMB) inhibition assay and immunoprecipitation with western blot analysis to examine the intracellular localization/storage of MPO and the molecular mechanisms underlying its release following IL-8 activation. We found that in addition to azurophilic granules, a much larger quantity of neutrophil MPO is pre-stored in the cell nucleus and released to the extracellular space following cell activation. Cell surface upregulation of MPO and release from the nucleus following neutrophil activation is regulated by the nuclear export molecule exportin 1. LMB (specific exportin1 inhibitor) inhibits IL-8 induced MPO release and cell surface upregulation. We conclude that the in human neutrophils the cell nucleus is the main source of MPO storage and release to the extracellular space. These results suggest a novel and prominent role for the neutrophil nucleus in inflammatory events related to the MPO-hydrogen peroxide-halide system.

Bone marrow transplant from HLA matched unrelated donors for severe combined immune deficiency

Grunebaum E, Atkinson A, Reid B and Roifman C.M. Division of Allergy and Clinical Immunology, Hospital for Sick Children

Introduction: Bone Marrow Transplant (BMT) from related HLA identical donor (RID) is the treatment of choice for patients with severe combined immune deficiency (SCID). In the absence of RID, HLA from haplo-

identical (half) related donors (HID) are often used. However HID is associated with reduced survival and long-term immune reconstitution failure. We have therefore used HLA matched unrelated donors (MUD) instead of HID in the past 15 years. *Methods:* We studied hematopoietic engraftment, graft versus host disease (GVHD) development, infections and long term survival in infants diagnosed with SCID that received MUD BMT between 1989 and 2004. Lymphocyte engraftment and detailed immune evaluation were performed in children that survived more than 2 years after transplant. *Results*: Twenty two infants underwent MUD BMT following myelo-ablative conditioning in a Canadian pediatric referral center specializing in such procedures. Molecular diagnosis was available in 64% of the patients. Donor lymphocyte engraftment, humoral and cellular immune function and T-cell repertoire were normal in all patients that were evaluated more than 2 years after transplant and off immuno-suppression, and they were sustained for more than 14 years of follow up. Acute graft versus host disease occurred in 18/22 patients, despite prophylaxis, and it was the most common cause of death. Overall, survival was 74% and was even better for patients with mutations in the IL-2 receptor pathway (5/6 surviving more than 6 years). Furthermore, survival of patients who presented with low B cells, previously estimated to have unfavorable outcome, was not significantly lower than in other forms of SCID in this study. *Conclusions:* MUD BMT leads to long term survival, engraftment and immune function in SCID patients and should be the preferred treatment for clinically stable infants that do not have a RID.

Article Categories:

- Annual Scientific Meeting

Treatment	*n* (%)
Total patients treated w/epinephrine	102 (79.1)
EpiPen (self-administered)	24 (18.6)
Epinephrine via ED personnel	86 (66.7)
IV access secured	76 (58.9)
IV fluid bolus	35 (27.1)
H_1 antagonists	122 (94.6)
H_2 antagonists	32 (24.8)
Corticosteroids	64 (49.6)
β2-Agonists	38 (29.5)
Admitted to hospital after initial mgt	8 (6.2)
Discharge Dx = anaphylaxis	53 (41.1)
EpiPen prescribed on discharge	76 (58.9)
Discharge therapy/adviceDischarge prescription including corticosteroids	37 (28.7)8 (6.2)

Question	Pre (%)	Post (%)	Change (%)	*p* Value
Comfortable with MD	93.9	96.9	3.1	.549
Confident to manage	58.2	89.8	31.6	<.001
Know what to do	60.2	98.0	37.8	<.001
Asthma medicine	75.5	99.0	23.5	<.001

Season Admitted	Alternaria +ve	Alternaria -ve
Fall	9	2
Winter	2	3

Published: 15 December 2006

doi:10.1186/1710-1492-2-4-1
Cite this article as: : Meeting program. *Allergy, Asthma & Clinical Immunology* 2006 **2**:1.