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Anaphylaxis after Topical Application of Bacitracin: A Case Report

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Introduction: We present a case of anaphylaxis following repeated application of bacitracin. **Method:** A case report of a twenty-three year old woman with no prior allergic history who underwent body piercing of the navel. A low grade local infection developed at the site and this was treated intermittently over a period of weeks with topical bacitracin (Baciguent). Following one application she experienced rapid onset of pruritus of the head and hands, generalized urticaria, dyspnea, wheeze and dizziness. She was successfully treated in hospital for anaphylaxis and released. **Results:** Skin prick testing with bacitracin yielded a 25 millimetre wheal with pseudopods. Testing of bacitracin on a control subject was negative. **Conclusion:** Body-piercing is a common cosmetic procedure. Localized infection at the site of piercing is a common complication. Repeated application of a potentially sensitizing agent to an area of inflamed skin enhances the potential for the development of sensitization. Bacitracin is a topical antibiotic with the capability of inducing anaphylactic sensitivity following topical application. In a survey of body-piercing establishments in Toronto, 75% recommended the use of over-the-counter topical antibiotics such as Bactrim or bacitracin to treat localized infections after piercing. The use of bacitracin at sites of inflamed body piercings poses a risk for the development of anaphylactic sensitivity. A history of bacitracin use should be determined in patients with body piercings who develop anaphylaxis.

Regulation in Expression of the High Affinity IgE Receptor (FcεRI) in Human Neutrophils

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Introduction: The expression of high affinity receptor for IgE, FcεRI is central for allergy and asthma. Previously we have shown that human neutrophils from asthmatics express FcεRI. In this study we investigated various factors that influence its regulation. **Methods:**

Peripheral blood neutrophils were isolated from adult atopic asthmatics ($n = 17$), atopic non asthmatics ($n = 15$) and non-allergic donors ($n = 16$) by dextran, ficoll gradient centrifugation and magnetic cell sorting (MACS). Surface, total protein and mRNA expression of FcεRI was investigated in the three groups by FACS, immunocytochemistry (ICC) and fluorescent in situ hybridization (FISH) respectively during the pollen allergic and outside the pollen season. Furthermore, neutrophils from atopic asthmatic subjects were stimulated in vitro with Th-2 cytokines (IL-4, IL-9, GM-CSF). Surface, total protein and mRNA expression of FcεRI by neutrophils was evaluated by FACS, Western blot, real-time PCR and FISH. **Results:** Neutrophils from atopic asthmatic subjects showed increased expression of FcεRI α chain in surface, total protein and mRNA compared to atopic non asthmatics and non-allergic donors ($n = 20$). In contrast to healthy donors and atopic non asthmatics ($n = 8$), FcεRI α chain surface and mRNA expression increased significantly during pollen season compared to non pollen season ($p = .001$) in neutrophils isolated from AA ($n = 9$). Interestingly, Th2 cytokines (IL-4, IL-9, GM-CSF) stimulated neutrophils ($n = 6$) at 6 and 18 hrs showed increased protein and mRNA expression of FcεRI α chain as assessed by FACS, Western blot, real time PCR, and FISH analysis. **Conclusion:** Our data suggest that the expression of FcεRI in neutrophils of atopic asthmatic patients is regulated in vivo. Furthermore it provides evidence that Th-2 cytokines such as IL-9, IL-4 and GM-CSF regulate the expression of FcεRI in neutrophils. Collectively neutrophil mediated FcεRI dependent activation may play a key role in allergic diseases.

Follow-up of Peanut Allergic Patients with Serum Peanut Specific IgE

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Background: Peanut allergy is not necessarily a lifelong problem. DBPCFCs (double-blinded placebo-controlled food challenges) are “gold standard” in diagnosing peanut allergy. In practice, peanut specific IgE (PN-IgE) are used to predict symptomatic allergy and

tolerance, and reduce the number of DBPCFCs. Current practice guideline is to do yearly measurement of PN-IgE. *Objective:* The goal of this study was to determine the optimal frequency of PN-IgE measurement as part of screening for development of clinical tolerance to ingested peanuts. *Method:* Retrospective review of charts of peanut allergic patients followed up and serially tested for PN-IgE with a quantitative antibody fluorescent-enzyme immunoassay at Laboratory of Immunology LHSC, from 1997-present. *Statistics:* Time to first decline of PN-IgE values was estimated using Kaplan-Meier technique. Group comparisons were made using log-rank statistics. *P* values less than .05 were considered statistically significant. *Results:* A total of 782 patients evaluated for food allergy were reviewed. Of those, 101 patients with peanut allergy fulfilled the study criteria (age at first reaction 6 mos-15 yrs old, median 1.5 yrs old; initial PN-IgE 0.4 - > 100 kU_A/L, median 18.5 kU_A/L). 12% and 63% of all patients achieved significant decrease in PN-IgE values after 2 and 5 years, respectively (median time was 41.7 months). Younger age (< 2 years old) at first reaction and first PN-IgE measurement predicted longer recovery time for PN-IgE (*p* = .002 and *p* = .016, respectively). At 2 years interval, 14.8%, 15.35% and 3.9% of patients with baseline values < 17.5 kU_A/L, 17.5-100 kU_A/L and > 100 kU_A/L respectively, had a significant decrease of PN-IgE values, whereas 5 year reduction rate was 49.69% and 80.4% of patients with baseline PN-IgE < 17.5 kU_A/L and 17.5-100 kU_A/L, respectively. Moreover, reduction rate of PN-IgE was determined by baseline values (*p* = .035). *Conclusions:* This study suggests that PN-IgE values can be measured at least every 5 years in order to predict the degree of tolerance and the results of DPCFCs. The decision to repeat PN-IgE varies upon the initial PN-IgE value. This study provides evidence for a possible new practice guideline for following up patients evaluated for peanut allergy.

The Intensity of Recall Cytokine Responses to Respiratory Viruses Associated with Allergic Status

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Respiratory syncytial virus, metapneumovirus (MPV) and reovirus are RNA viruses that commonly infect humans. RSV and MPV have an apparent association with asthma exacerbations. We hypothesized that these

ubiquitous viruses elicit potent recall responses dominated by Th1-biased cytokine production, and that the intensity of such recall cytokine responses associates with clinical status. We established short-term primary culture systems using peripheral blood mononuclear cells (PBMC) from adults to evaluate (i) the prevalence of virus-specific cytokine recall responses, (ii) virus-induced cytokine producing cell subsets, (iii) to test the hypothesis that different recall responses are evident to respiratory viruses in mildly asthmatic versus non-atopic humans. Fresh PBMC from > 60 individuals were isolated and cultured with live virus. Supernatants were harvested 1-6 days later, with the frequency and intensity of type 1 (IFN_γ), type 2 (IL-13, IL-5) and IL-10 virus-specific responses quantified by ELISA. Virus-specific IFN_γ was dependent on classical T cell activation, as demonstrated by costimulatory blocking cultures and flow cytometry analysis. Interestingly, reovirus-specific IFN_γ responses were stronger in asthmatic and allergic individuals compared to non-atopics (*p* < .001). These differences were not apparent in MPV and RSV-specific IFN_γ responses. In summary, these respiratory viruses provide a powerful model for examination of the impact of viral infections in modulating established human immune responses to environmental allergens. *Support:* CIHR, Canada Research Chair in Immune Regulation, Tom and Mindel Olenick Award in Immunology, Manitoba Health Research Council.

VAMP-7 and VAMP-2 Are Potential Partners for Syntaxin-4 and SNAP-23 during Exocytosis in Human Basophils and Mast Cell

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Background: Mast cells and basophils, critically important granulocytes in allergic reactions and asthma, synthesize and secrete a wide variety of proinflammatory mediators. Products such as vasoactive amines, proteases, cytokines and chemokines are thought to contribute to tissue injury and remodeling. Different isoforms of SNARE (soluble NSF attachment protein receptors) proteins have been implicated in regulation granule and vesicle docking during exocytosis by some leukocytes. However, little is known about the mechanism(s) leading to membrane docking and fusion in basophils. *Objective:* We investigated the expression of SNARE proteins using both peripheral blood-derived human basophils and a basophilic cell line, KU-812. We compared our findings with SNARE profiles from two mast cell lines: human mast cell-1 (HMC-1) and the Laboratory of Allergic Diseases-2 (LAD-2). *Methods:* Highly purified human peripheral blood basophils (≥ 96%) from atopic subjects were obtained by nega-

tive immunomagnetic selection. Total nucleic acid (RNA) was extracted and subjected to RT-PCR. Western blot analysis and confocal microscopy were also used to identify protein expression and distribution of distinct isoforms of SNARE complex. *Results:* Human basophils ($n = 5$) expressed mRNA for the v-SNARE isoforms, VAMP (vesicle-associated membrane protein)-1, -2, -3, -7 and -8 and the t-SNAREs SNAP-23 and syntaxin -3, -4 and -6. KU-812, HMC-1 and LAD-2 shared the same SNARE phenotype profile with those in human basophils. Syntaxin-3, -4, -6 and SNAP-23 and VAMP-7, VAMP-8 were also identified at the protein level using Western blot analysis of human basophils. Confocal imaging showed the substantial colocalization of v-SNARE, VAMP-2 and VAMP-7 with CD-63 (granular marker). *Conclusions:* Our findings suggest that human basophils express SNARE isoforms necessary for docking of their granules and secretory vesicles during exocytotic processes leading to mediator secretion.

The Incidence of Drug Hypersensitivity and Idiosyncrasy as a Presenting Problem to an Emergency Department

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Background: The community incidence of allergic reactivity to medications is varied, but known to be less than that observed in inpatient populations. Outpatient rates of 0.06%, 0.13%, and 0.30% have been reported from Australia, Italy, and the U.S., respectively. *Objective:* To describe the outpatient epidemiology of drug hypersensitivity occurring in a tertiary care center in Canada. *Methods:* Three years of emergency department (ED) charts were reviewed. All ED visits given a discharge diagnosis of "allergic reaction" or "anaphylaxis" were pulled and directed to the investigator. Chart review and direct patient contact determined if criteria for these diagnoses were properly met. *Results:* Over the 3-year time period, 153,990 patients were assessed in the ED at KGH. A total of 554 cases of "allergic reactions" (including anaphylaxis) were identified. Of these, 111 were labeled secondary to medications. Further chart review reduced this number to 101 reactions that could be classified as either allergic or idiosyncratic (pseudo-allergy). This yielded a 0.07% incidence of drug hypersensitivity/idiosyncrasy as a presenting problem. 22 (21.8%) of the reactions were anaphylactic in nature, 3 required admission to hospital, there were no fatalities. Antibiotics were the most commonly implicated medication, accounting for 57 (56.4%) of all reactions. Of the antibiotics, penicillins (21), macrolides (11) and sulfonamides (9) were most frequently involved. NSAID idiosyncrasy accounted for 15 (14.9%) of the reactions. Opiates followed with

8 (7.9%). *Conclusion:* Drug hypersensitivity/idiosyncrasy is an important presenting problem to emergency departments in Canada and elsewhere. The incidence in this study was 0.07%, mostly to antibiotics. This study was internally funded. The author would like to acknowledge Dr. James Day for his editorial review of this abstract.

Delayed Hypersensitivity to Titanium Causing Pacemaker Allergy

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Background: Component allergy is an extremely rare, but documented complication of pacemaker insertion. *Case:* A 33-y-old male with a 28-y history of pacemaker dependence presented to cardiology complaining of pain and redness at his pacemaker site. He had a history of recurrent pocket site infections leading to the replacement of multiple transthoracic, and later transvenous pacing systems, the most recent having been inserted 3 years ago. He also had a history of atopy including anaphylaxis to latex, kiwi, and methylparaben. Approximately 2 months prior, he developed tenderness, swelling and redness overlying his pacer site. Examination revealed erythema and minimal edema, but no localized heat. He was afebrile. Multiple blood cultures were negative. No pacemaker pocket fluid or other abnormalities were identified with ultrasound or computed-tomography scanning. A 5-day course of prednisone produced partial relief, but symptoms recurred. Empiric courses of ciprofloxacin and carbamazepine yielded no response. He ultimately required admission to hospital 5 months into his illness for intravenous opiates to achieve pain control. Repeat investigations were unremarkable, including an ESR of 1 mm/hr. Antibiotics were discontinued with no worsening of symptoms. An allergy consultation led to patch testing to the four components of the device (titanium, polyurethane, silicone, and polyurethane insulation) resulting in a positive reaction to titanium only. Titanium patch testing on a non-allergic control subject was negative. The patient was diagnosed with delayed hypersensitivity to the titanium-coated pacemaker. His pacemaker was removed and a gold-coated replacement system was inserted. Cultures from the original pacer were negative. The patient has done well subsequently, having no evidence of reactivity at the site. *Conclusion:* Delayed hypersensitivity to titanium-coated pacemakers can result in chronic localized inflammation and apparent recurrent infections at the site of implantation which can be misleading. Persistent reactivity at the site of pacer implantation is reason to suspect this rare condition.

Medicine Wheel Navigates Sage Journey

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SAGE, funded by CIHR, is a Manitoba Institute of Child Health, University of Manitoba project designed to Study in Asthma the role of Genes and the Environment in the 1995 Manitoba birth cohort. One thousand children and their families from across the province will be enrolled in this project, 200 children from rural Manitoba, 200 from First Nation's communities. The First Nation's children have a high incidence of respiratory disease (not necessarily asthma) and provide a unique opportunity to study a genetically diverse population living in both developed and developing world environments. The Medicine Wheel is used as the navigation tool to achieve a balance in the overall process from both parties involved in the SAGE project. First Quadrant of the Medicine Wheel emphasizes the inherent rights as First Nations people before and after colonialism and colonization and secondly, how First Nations connect the ownership in relation to research. Second Quadrant focuses on the importance and significance of the First Nation people's autonomy and taking control of their lives in all aspects: physically, emotionally, mentally and spiritually and secondly, how First Nations can relate to control in relation to research data and reports. Third Quadrant shares the SAGE journey and its correlation of a personal journey centered on trust and openness. The gaps and strengths of SAGE are shared and the ways on how the whole process was navigated. Fourth Quadrant teaches the importance of involving the whole community from the children, youth, adults, and the elders. The appropriate research practices on partnership building, developing linkages and the decision-making practices are included to stress unity and equality.

T_H2 Involvement in Peanut Antigen-Specific Responses: Comparison between Adult and Juvenile Populations

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Peanut allergy is one of the most severe food allergies, representing an important cause of food induced anaphylaxis or fatality. While extensive investigation has been made of allergen structure and the nature of antibody recognition of peanut allergens, little is known about the role played by T cells in the maintenance of tolerance vs allergy to peanut. Here, we developed systems to examine human peanut allergen specific

T cell cytokine responses and investigate differences between (i) peanut allergic, (ii) peanut sensitized (skin test positive but clinically non-atopic) and (iii) peanut non-sensitized individuals. Using a primary culture system we developed in which freshly isolated human PBMC are stimulated with whole peanut allergen extract, we compared patterns of responsiveness in adults (60 adults, 18-40 years old) and children (40 8-9 year olds) by assessing the frequency and nature of responses characteristic of Th1-like (IFN γ , CXCL10) and Th2-like (IL-5, IL-13, CCL17, CCL22) recall responses to peanut allergen. Among adults, we found that peanut specific Th2 responses were readily demonstrable in ~ 50% of non-atopic/non-sensitized individuals and 90% of sensitized (but clinically unresponsive) subjects. These were CD4 T cell dependent and required classical T cell activation as demonstrated by the capacity of α HLA-DR, α CD80/86 or CTLA4-Ig to block recall responses. In contrast, IFN γ and CXCL10 responses were rarely seen. Thus, analysis of CD4 T cell dependent allergen specific responses demonstrates that (i) a substantial proportion of clinically asymptomatic humans exhibit immune responses to this common food antigen, enabling us to investigate the mechanisms underlying the maintenance of food allergy and (ii) Th1-like responses, characteristic of protection from allergic disease to inhalant allergens, are extremely rare. Current studies are aimed at determining the contributions such immunity plays in shaping the food allergy vs tolerance decision. *Support:* CIHR, NSERC Studentship, Manitoba Institute for Child Health Studentship, Canada Research Chair in Immune Regulation.

Case Report:

Drug-Induced Eosinophilic Myocarditis

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A 50-year-old gentleman presented with class III NYHA heart failure associated with malaise and low-grade fever. He had increased migraine headaches in the preceding one month. During this period he was taking sumatriptan (Imitrex) for approximately 4 days per week and ibuprofen daily. He denied taking other medications. He did not have any recent travels. He does not have a history of asthma or sinusitis. His physical examination was consistent with congestive heart failure. There were no rashes. The ECG showed changes suggestive of ischemia. There was a mild rise in troponin level. White blood cell count was elevated at 15.1 g/L (4.0-11.0 g/L) with increased neutrophils at 13.4 g/L (2.3-7.7 g/L). Peripheral eosinophil count was 0. Blood work from 4 weeks prior to presentation indicated a normal WBC with 0 eosinophils. Blood tests were

negative for ANA, rheumatoid factor, ANCA, cryoglobulins, HIV serology, and hepatitis B serology. CT scan of the head was normal and CSF analysis was normal. Echocardiogram showed left ventricular hypertrophy and an ejection fraction of 45%. Coronary angiography revealed normal coronary arteries. Endomyocardial biopsy revealed necrotizing myocarditis with prominent eosinophil infiltrate. The sumatriptan and non-steroidal anti-inflammatory drugs were discontinued. He was started on prednisone 60 mg daily which after 2 months was tapered down to 15 mg daily. He was also initially treated with furosemide, ramipril and carvedilol. Echocardiogram 3 months after initial presentation showed a normal ejection fraction. The furosemide and ramipril were discontinued. Peripheral eosinophil count has remained normal. This is a unique case of eosinophilic myocarditis potentially associated with sumatriptan and/or non-steroidal anti-inflammatory drugs.

Socioeconomic Status and the Development of Asthma

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The association between low socioeconomic status and asthma is inconsistent, and not compatible with the hygiene hypothesis. We undertook this research to determine the relationship between socioeconomic status and the development of asthma, and to identify environmental factors that may explain this relationship. In 2002/03, a survey was sent to 12,556 households of children born in Manitoba in 1995, asking parents whether they or their 7-year old child had asthma, and whether smokers or pets were present in the birth home. Survey responses were linked to health care records for lower respiratory tract infections (LRI) during infancy, and to a census-based measure of income. The likelihood (odds ratio, OR) of asthma was determined according to income, and exposure to tobacco smoke, pets and LRI within the first year of life. 3,564 (28.4%) of the surveys were returned. Parents reported asthma in 12% of children living in urban areas. Asthma prevalence declined progressively by income area from 17% to 11% in the highest income neighbourhoods. Asthma prevalence was not related to income in rural areas. Lower income, urban children were more likely to be exposed to tobacco smoke, cats and LRI during infancy. In comparison to high income, urban children, the odds ratio for asthma in low and middle income children was 1.74 (95% CI: 1.07–2.83) and 1.32 (95% CI: 0.98–1.78), respectively. The increased likelihood of asthma in lower income children was independent of family history of asthma/allergy (OR = 3.93, 95% CI: 2.93–5.28) and of exposure to LRI

in infancy (OR = 3.29, 95% CI: 1.37–7.88). The income-asthma association in urban children was not diminished by early childhood exposure to tobacco smoke, cats or dogs. An inverse association between the development of asthma and socioeconomic status was only observed in Manitoba children living in urban areas. This association was not explained by early exposure to household allergens.

The Natural History of Wheezing Syndromes in Pre-Term Children

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Rationale: To describe the natural history of wheezing syndromes in premature/low birthweight babies compared to term/normal birthweight babies. *Methods:* The Manitoba Health Services Insurance Plan (MHSIP) database is a population-based, health care administrative and prescription database. It has records of every child born in 1995 and subsequent utilization of the provincial health care system. The number of children diagnosed with a wheezing syndrome (defined as an ICD-9 code of 493 [asthma diagnosis for hospitalization or physician visit] or a prescription of an asthma medication) was obtained. The relative risks (RRs) of wheezing in premature/low birthweight children compared to term/normal birthweight were determined up to 7 years of age. *Results:* 13,980 children were born in 1995 and are currently living in the province of Manitoba. In comparison to term infants (37–42 weeks gestational age [GA]), the RRs of a wheezing syndrome in infants born < 28 weeks GA ($n = 45$) at 1, 3, 5, and 7 years were 1.76*, 1.44, 1.75* and 1.30. RRs for 28–32 weeks GA ($n = 84$) were 1.72*, 2.39*, 2.26* and 1.25* and for 32–37 weeks GA ($n = 763$), RRs were 1.17*, 1.14, 1.14, and 1.25* at 1, 3, 5, and 7 years. In comparison to normal birthweight infants (2,500–4,500 g), the RRs of a wheezing syndrome in infants born < 1,000 g ($n = 37$) at 1, 3, 5, and 7 years were 2.25*, 2.12*, 2.14* and 1.35. In babies with birthweights between 1,000–1,500 g ($n = 70$), RRs were 1.52*, 1.94*, 1.92* and 1.55 at 1, 3, 5, and 7 years. *Conclusion:* Babies born at low gestational ages and with low birthweights have an increased risk of a wheezing syndrome in the first seven years of life.

*Denotes statistical significance.

Cytokine Responses to Toll-Like Receptor Stimulation in Children

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Introduction: Activation of Toll-like receptors (TLRs) by distinct microbial ligands triggers not only innate immunity, but also regulates the nature of the adaptive immune response, and thus may influence either devel-

opment of, or exacerbation of, allergic diseases such as asthma. The role of TLR responsiveness in human asthma remains unknown. We hypothesize that functional responsiveness to TLR stimulation by physiologically relevant ligands differs in asthmatic and healthy control children. The specific objective of this study has been (i) to identify “optimal” and “threshold” doses of a panel of relevant TLR ligands and (ii) to determine optimal experimental conditions for quantitative analysis of patterns of cytokine and chemokine responses. *Methods:* PBMC obtained from ~ 200 8-9 year old children were stimulated with distinct TLR ligands: lipopolysaccharide, peptidoglycan, 3M-011 compound or poly(I:C) at concentrations over five log range for 24 h. This time point was chosen on the basis of kinetic studies. Absence of endotoxin contamination was verified by LAL assay. Levels of cytokines and chemokines produced by these children were measured by ELISA. *Results:* All individuals studied responded to these TLR ligands. The “optimal” and “threshold” conditions were identified. Specifically, lipopolysaccharide and peptidoglycan have the ability to stimulate production of cytokines (IL-6, TNF- α , IL-1 β , IL-12p40, IL-10) and chemokines (CCL2, CCL22) in a dose dependent manner. 3M-011 elicits a similar pattern of cytokine responses and induces significant levels of IFN- α and CXCL10. Stimulation with poly(I:C) resulted in production of IFN- α and CCL2; levels of other tested cytokines and chemokines were below the limit of detection for these ligands. *Conclusion:* Lipopolysaccharide, a TLR4 ligand, peptidoglycan, a TLR2 ligand, 3M-011, a TLR7 ligand, are potent stimuli of cytokine and chemokine production by PBMC derived from children. Poly(I:C), a TLR3 ligand, induced production of IFN- α and CCL2, but few other cytokines. “Optimal” and “threshold” stimulation concentrations of lipopolysaccharide, peptidoglycan, 3M-011 and poly(I:C) were identified that will be used to test the main hypothesis upon unblinding of the study. *Research support:* SAGE, Canadian Institutes of Health Research, CRC Chair Program.

Peptide-Based Vaccines against Human Interleukin-13 (hIL-13)

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IL-13 is one of the key contributors in allergic asthma. It promotes IgE class switching and in the lung upregulates eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness. Immunologically down-regulating the level of IL-13 may be a better approach for asthma treatment than the current pharmaceutical therapies. This is supported by the successful admin-

istration of humanized monoclonal antibodies to IgE as passive immunotherapy in asthma treatment. However, a short half-life and extremely high cost limit the use of this approach. To overcome these disadvantages, we aimed to develop active immunotherapy using an anti-hIL-13 vaccine which induced auto-neutralizing antibodies to hIL-13. The vaccine conjugate consisted of a hIL-13 peptide (12-17 amino acid residues) derived from hIL-13 receptor binding sites, made immunogenic by linking it to a foreign carrier protein via two approaches. (1) Chemical methods: synthesized peptides were coupled to bovine serum albumin (BSA) using the glutaraldehyde method; (2) molecular engineering: the peptide was fused to the hepatitis B core antigen (HBcAg) to form chimeric HBcAg. Three chemically coupled conjugates and one chimeric HBcAg that presents as capsule-like particles were tested in mice. Mice were immunized three times with a vaccine conjugate emulsified in an adjuvant. Immunization with the carrier BSA or natural HBcAg served as controls. Sera were collected two weeks after the last immunization. Titres to hIL-13 were measured by an ELISA. Two vaccine conjugates and the chimeric HBcAg were found to elicit high titres of antibodies to hIL-13. Inhibition tests revealed that these vaccinated sera inhibited the binding of hIL-13 to its receptors in a dose-dependent manner using receptor-capture ELISA and hIL-13- induced B cell proliferation tests, suggesting that vaccine-induced antibodies are able to inactivate hIL-13 in vitro. Studies of the in vivo effect of the vaccine are currently in progress in a mouse model of asthma. This study was supported by The Hospital for Sick Children Foundation (Toronto) and the Children’s Hospital Foundation of Manitoba Inc.

Is There an Association between Childhood Immunizations and Childhood Asthma?

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Background: The prevalence of childhood asthma has steadily increased over the past two decades. This is of great concern as the implications of this have been huge both socially and economically (direct and indirect costs). There are many theories as to why there has been such an increase in asthma and allergies in the developed world. One such theory is the Hygiene Hypothesis. This particular theory has provided the background for my current research. It states that our immune systems have not been able to adapt quickly enough to the rapidly occurring changes in our living environments (improved sanitation, new and improved antibiotics and immunizations). Thus, these changes have made it much easier for our immune systems to slip into unbalanced states where allergies and asthma can arise.¹ *Methods:* My thesis is a retrospective birth

cohort of Manitoba children born in 1995. My outcome of "asthma" has been validated by Dr. Anita Kozyrskyj and Dr. Allen Becker in their NET study. I have been linking immunization data from the Manitoba Immunization Monitoring System (MIMS) to other administrative data such as drug, medical and hospital data using SAS (Statistical Analyses Software). I am particularly interested with the diphtheria combination vaccines (both cellular and acellular), the measles/mumps/rubella (MMR), as well as the BCG vaccine. I am analyzing the types of vaccines which are given, the time frame in which they are given, the children's immunization status, and their possible association with asthma. *Results:* My results and the statistical relevance of my findings have yet to be completed. However, as an example of my preliminary findings on the 13,980 children in the cohort I have found the following: 1,569 of the children have been determined to have asthma at age 7 with the onset varying from 0 to 7 years of age. Only 416 children were vaccinated with BCG, of which 33 had asthma (7.93%) compared to an asthma rate of 11.32% in the rest of the population. Of the children in the cohort 97.5% of them had had one or more MMR vaccines, while only 110 children or 0.79% had never received any immunizations. *Conclusion:* It is premature to state my final conclusions for the specific questions raised in my thesis. However, regardless of the final outcome I believe that this research is timely and will be of value to the scientific community.

Human Eosinophils Constitutively Express Indoleamine 2,3-Dioxygenase and Regulate T-Cell Function

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Indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan to kynurenines (KYN), which in turn inhibit proliferation and survival of T cells, especially Th1. Regulatory dendritic and T-cells downregulate T-cell function via KYN-mediated mechanisms. Eosinophils, whose numbers increase following allergen challenge, have the potential to interact with T-cells. We hypothesized that increase in IDO-expressing eosinophils during allergic inflammation is an immunoregulatory mechanism in the maintenance of -Th2 polarization in allergic inflammation. Eosinophils from atopic and non-atopic donors were probed for IDO expression using RT-PCR and Western blotting. The effect of IL-3, IL-5 and GM-CSF on IDO expression was determined using quantitative PCR. KYN was measured to determine enzymatic activity of IDO. Eosinophils were co-cultured with IFN γ - or IL-4-producing T cell lines

or clones before measuring apoptosis or proliferation of T cells. IDO expression in lung tissue of allergic subjects, and in tissues from a mouse model of allergic inflammation, was examined using immunohistochemistry. Eosinophils express IDO mRNA and protein constitutively and produce KYN following IL-3, IL-5, GM-CSF, and IFN γ treatment, leading to T-cell apoptosis and inhibition of PHA-induced proliferation of PBLs. While IL-3 reduced IFN γ -induced IDO mRNA below resting levels in Eos, IL-5 induced only a 2-fold reduction. Conversely, treatment with GM-CSF led to a 3-fold increase in IFN γ -induced mRNA expression. Crosslinking of CD28 on eosinophils induced IFN γ release with autocrine effects, leading to tryptophan catabolism to KYN. Co-culture of T cells with eosinophils inhibited proliferation of an IFN γ -producing T cell line but not an IL-4-producing T cell clone. There was extensive infiltration of IDO-expressing eosinophils into lymphoid aggregates from atopic subjects. Eosinophils were the main IDO-expressing cells found in the lung tissues of OVA-sensitized mouse model of allergic inflammation. Our data suggest that eosinophils may potentially regulate T-cell function in vivo through IDO-dependent pathways, thus contributing to the maintenance of Th2 polarization characteristic of atopic disease.

Interleukin-17 Modulates IL-1 β Induction of IL-8 and IL-6 Expression in Human Airway Smooth Muscle Cells: Role in Neutrophilic Inflammation

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Background: Airway neutrophilia is a predominant feature of acute lung disorders such as chronic obstructive pulmonary disease (COPD) and severe asthma. While IL-17 induced expression of the CXC chemokines in the airways leading to neutrophil recruitment is well established, potential direct effects of IL-17 on airway smooth muscle (ASM) function have not been determined. *Aim:* This study aimed to investigate the role of IL-17R in the activation of human ASM cells and release of inflammatory mediators. *Experimental Procedures:* IL-17R mRNA and surface bound receptor expression were investigated by RT-PCR and flow cytometry. Immunofluorescence study was carried out to detect IL-17R within bronchial smooth muscle. ELISA and quantitative real-time PCR were carried out to investigate the effect of IL-17 and IL-1 β stimulation on IL-8 and IL-6 mRNA and protein expression. In vitro chemotaxis assay measured the effect of conditioned medium of IL-17 stimulated ASM cells on the migratory capacity of human neutrophils. In vivo expression

of IL-17R in human ASM bundle within bronchial sections of COPD patients was performed by immunofluorescence coupled to confocal microscopy. *Results:* ASM cells expressed steady state of IL-17R protein, mRNA and surface bound receptor. IL-17 stimulated IL-8 and IL-6 release from ASM cells in a time- and dose-dependent manner that was significantly inhibited by neutralizing anti-IL-17 mAb. Interestingly, IL-17 dramatically enhanced IL-1 β induced expression of IL-6 and IL-8 protein and mRNA. The effect of IL-17, alone or in combination with IL-1 β , was abrogated by actinomycin-D, suggesting that IL-17 regulates IL-6 and IL-8 at the transcriptional level. In vitro chemotaxis assay showed that IL-17 induced IL-8 release from ASM cell conditioned medium attracted neutrophils. Finally, ASM cells bundle within human airway sections from COPD patients showed IL-17R positive immunostaining. *Conclusion:* These data clearly demonstrate that ASM cells are a target for IL-17 and suggest that ASM cells participate via an IL-17 dependent manner in the recruitment of inflammatory cells, particularly neutrophils, into the airway.

Syk Tyrosine Kinase Participates in β 1 Integrin Signaling and Inflammatory Responses in Airway Epithelial Cells

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The protein tyrosine kinase Syk is best known as a critical component of immunoreceptor signaling complexes in hematopoietic cells. Recent studies showed Syk expression in some nonhematopoietic cells, implicating its involvement in other important cellular functions. We have recently demonstrated that Syk is widely expressed in respiratory epithelial cells (EC) in situ, as well as in cultured primary bronchial EC and cell lines HS-24 and BEAS-2B. We hypothesized that Syk functions as a signaling molecule involved in inflammatory responses in the epithelium. To characterize Syk expression in airway EC, immunohistochemistry, Western blot, PCR, and laser scanning confocal microscopy were used. Syk-dependent signaling pathways in EC were initiated by engagement of β 1 integrin receptors. Stimulation of β 1 integrin receptors by fibronectin or antibody cross-linking caused redistribution of Syk from a cytoplasmic to plasma membrane localization. In stimulated cells, Syk and integrin β 1 co-localized. In addition, following β 1 integrin receptor engagement, tyrosine phosphorylation of Syk was observed. Expression of the adhesion molecule ICAM-1 and production of IL-6, both important molecules in lung inflammation, was down-regulated in EC treated with

Syk small interfering RNA, or Syk inhibitor piceatanol. We propose that Syk is involved in signaling pathways induced by integrin engagement in airway EC. Syk-mediated signaling regulates IL-6 and ICAM-1 expression and may be important in the pathophysiology of lung inflammation. Funded by CIHR, CSACI/CAAIF/Merck Frosst, Alberta Heritage Foundation for Medical Research, and NIH.

Anaphylactic Reaction during Intrauterine Insemination Caused by Egg Yolk Allergy

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Introduction: Anaphylaxis during artificial insemination usually occurs with the following allergens: latex, human spermatic fluid, drugs added to the sperm processing media, and the preservative bovine serum albumin (BSA). *Case History:* This 27 year old woman with allergic rhinoconjunctivitis and asthma has had 10 attempts of artificial insemination since 1999. After each injection of thawed semen, she experienced within a few minutes severe lower abdominal pain accompanied by pallor and malaise. These episodes were thought to be vaso-vagal reactions. On the tenth attempt, she immediately developed generalized pruritus, urticaria, angioedema, vomiting, respiratory distress and hypotension. After standard emergency treatment, she recovered without complications. All artificial inseminations use sperm processed in a solution supplemented by sterile egg yolk. Further history revealed that the patient never had an anaphylactic food or drug reaction. However, during the past 5 years she could not eat raw eggs because of a tingling sensation in her mouth and a tight throat. She tolerates baked goods containing eggs and cooked eggs. The skin prick tests were positive for egg white (4 mm of induration) and egg yolk (8 mm), and the specific IgE level was slightly positive (0.43 KUA/L) for egg yolk. Testing was negative for latex and spermatic fluid. Finally, she had an uneventful artificial insemination using sperm preserved with BSA three months after the anaphylactic reaction. *Conclusion:* We describe the first case of a life threatening anaphylaxis in an egg allergic woman who underwent an artificial insemination using sperm processed in fresh egg yolk. Even if egg allergy is extremely uncommon in adults, enquiry about food allergies remains an important part of all medical histories and particularly before prescribing injectable medicines or biological products.

Successful Treatment with Subcutaneous Immunoglobulin Infusion in a Primary Immunodeficiency Patient with Severe Adverse Reactions to Intravenous Immunoglobulin

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Background: Intravenous immunoglobulin (IVIG) infusion is an effective treatment for children with primary immunodeficiencies but it requires administration in a hospital setting and can be complicated by systemic reactions or poor venous access. Subcutaneous immunoglobulin (SCIG) infusion is an alternate treatment option. It is as effective as IVIG with no reported life-threatening systemic side effects and can be administered at home. **Case History:** A 5 year old girl with DiGeorge syndrome and hypogammaglobulinemia was referred because of severe adverse reactions to IVIG. She had features of DiGeorge including a vascular ring, speech delay, high arched palate, distinct facial features and combined immunodeficiency. She was positive for the chromosome 22 deletion. She had recurrent infections complicated by bronchiectasis, low antibody levels and poor specific responses to her vaccines. She was started on IVIG. She suffered severe headaches, vomiting, pallor and one episode of loss of consciousness 48 hours after several of the infusions of IVIG. Pretreatments with Benadryl, Tylenol, corticosteroids either immediately before or for 3 days following IVIG were all ineffective. Trial of different IVIG preparations did not change frequency or severity of adverse reactions. We elected to give her a trial of SCIG. The first infusion without any premedication had an uneventful course. She received 10 cc of Behring immunoglobulin (16%) solution over 1 hour given by syringe pump into her right thigh. She developed a local painless swelling at the injection site, which resolved over 2 hours. She subsequently received a total of three uneventful infusions in hospital, and then was discharged to continue outpatient treatment at her local hospital. **Conclusion:** We describe the first case in Canada of a primary immunodeficient child with adverse reactions temporally related to IVIG who tolerated treatment with SCIG. This case demonstrates that SCIG is

a suitable treatment option for patients with adverse reactions to IVIG.

Different Antigens with and without Adjuvant in the Induction of Airway Inflammation and IgE and IgG Responses in Mice

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Background: Mouse models of allergic asthma with ovalbumin (OVA) in alum have been used for many years. However, OVA is not a common allergen to humans and using alum for mouse sensitization is not close to human allergic diseases. **Objective:** To evaluate the effect of different antigens and the use of natural allergens without alum in the induction of IgE and IgG responses and airway inflammation in mice. **Methods:** Mice were intraperitoneal (i.p.) sensitized and intranasal (i.n.) challenged as below: A: sensitized twice with 2 µg of OVA in alum at weeks 0 and 2 and challenged (50 µg of OVA) at week 4; B: sensitized with 10 µg of OVA twice a week for 4 weeks, and challenged at week 5; C: the same as protocol "A," except that OVA was replaced with 100 µg of ragweed; D: the same as protocol "B," except that OVA was replaced with ragweed. Sera were obtained every 2 weeks, and bronchoalveolar lavage fluids (BALFs) were collected 1 week after the nasal challenge. Serum total IgE, OVA- or ragweed-specific IgE and IgG2a levels were measured by ELISAs. BALF eosinophils were stained and counted. **Results:** Mice sensitized with OVA in alum had the highest total IgE level followed by groups B, C and D (mean OD₄₁₀ = 3.39, 2.11, 1.42, and 1.12 for groups A, B, C, and D, respectively). Antigen-specific IgE levels were higher and specific IgG2a levels were lower in groups with alum than groups without alum ($p < .01$). There is no significant difference of eosinophilic percentages in OVA groups with or without alum (60% for A and 59% for B), which were higher than ragweed groups (24% for C and 41% for D). **Conclusion:** OVA induces higher IgE and eosinophilic responses in BALB/c mice than ragweed. Injections of natural antigens without alum are able to induce IgE and airway inflammation responses. This study was supported by The Hospital for Sick Children Foundation (Toronto).

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