7th BIENNIAL SYMPOSIUM of the INTERNATIONAL EOSINOPHIL SOCIETY, INC.

Eosinophils 2011 FOREVER YOUNG

JUNE 21-25, 2011
FAIRMONT LE CHÂTEAU FRONTENAC
QUÈBEC CITY QUÈBEC CANADA

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WELCOME TO QUÉBEC CITY

Samuel de Champlain founded Québec City (pronounce KEBEK) on July 3, 1608. In Algonquian (the spoken language of one of the native nations living in the area at the time), Québec means place where the river narrows. The old City is part of the Unesco world heritage.

For numerous years, Québec City was a key Settlement in North America and was heavily militarized. You will notice fortifications (city walls) surrounding the old City that are reminiscent of its strategic importance. Other key sites such as the Plains of Abraham and the Citadel are also important witnesses of the long lasting military presence in the City.

What to visit in the City
Take advantage of your stay to walk the old City notably “Place Royale” and the “Petit Champlain,” walk “la rue Saint-Jean,” “la place d’Youville,” and “la rue Saint-Paul.” Don’t hesitate to take the ferry across the St-Lawrence River in order to have a look at the old City from another point of view. The Fairmont organizes tours and a Tourist Office is located in the front of the Château. Additionally, you will find key information about what to do in Québec at the following website: www.quebecregion.com/en

Shows and events
There are many artistic events during the summer. At dusk, go the “Vieux Port” to watch the The Image Mill (le moulin à images; www.lacaserne.net/index2.php/other_projects/the_image_mill/) projected on the harbor building (free). Attend the “Fêtes de la St-Jean”, a music show on the “Plaines d’Abraham” followed by fireworks during the evening of June 23. Reserve a seat for the “Cirque du Soleil.”
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ACKNOWLEDGEMENTS

We would like to thank the following corporations, institutions, foundations, societies and individuals who contributed to the Symposium:

SYMPOSIUM SUPPORTERS

Platinum ($50,000+)
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Johns Hopkins University
McMaster University

We thank the following individual for his outstanding fundraising efforts on behalf of the IES.
Steven Ackerman

We thank the following individuals for their diligent evaluation of the abstracts:
Helene Rosenberg, Paige Lacy, David Abraham, and Michael Minnicozzi

We thank the following individuals for their service on the award committees:
Seema Aceves, Steven Ackerman, Monique Capron, Kimberly Dyer, Allison Fryer, Gerald Gleich, Simon Hogan, Francesca-Levi Schaffer, Redwan Moqbel, Per Venge, and Andrew Wardlaw
David Bass

We recognize with sadness the death of Dr. David Bass, a dedicated eosinophilologist. David died in July, 2009 after a long illness. At his death, David A. Bass, M.D., D. Phil., was Professor Emeritus of Internal Medicine at Wake Forest University School of Medicine. During his 30-year career in the Department of Internal Medicine, Dr. Bass served with distinction as the Thomas H. Davis Professor of Pulmonary Medicine, and in many capacities including Chief of the Section on Pulmonary, Critical Care, Allergy and Immunology, Acting Chief of Rheumatology, and Co-Head of the Section on Molecular Medicine. He also directed the MD/PhD program at Wake Forest University. Dr. Bass was an outstanding investigator, physician-scientist and mentor. Because of his research accomplishments and international reputation, Dr. Bass was elected to the American Society of Clinical Investigation, and received a number of other prestigious honors throughout his productive career. David’s initial studies of eosinophils were undertaken in concert with his training with the late Professor Paul Beeson at Oxford University. Professor Beeson, as an savvy physician-scientist, and his research group at Oxford University made significant and salient contributions to understanding the immunobiology of eosinophils, including studies documenting the T cell dependence of eosinophilia. David Bass made contributions to understanding mechanisms of eosinopenia in inflammation and of leukocyte activation and co-authored in 1977 with Professor Beeson a monograph on eosinophilia.
**SCIENTIFIC PROGRAM**

**TUESDAY, JUNE 21**

**Welcome Reception** - 17:00 to 19:00

**WEDNESDAY, JUNE 22**

**Breakfast** (on your own)

Peace symbol All sessions take place in the Frontenac Ballroom

**Presidential Address** - 09:00 to 09:15

James Lee (USA)

**Introduction - The Eosinophil Ambassadors** - 09:15 to 10:00

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<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tbody>
<tr>
<td>09:15</td>
<td>What lies before our eyes may be lies: eosinophil degranulation---in the absence of intact eosinophils</td>
<td>Gerald Gleich (USA)</td>
</tr>
<tr>
<td>09:30</td>
<td>Of mice and men - ECP makes the difference?</td>
<td>Per Venge (Sweden)</td>
</tr>
<tr>
<td>09:45</td>
<td>Eosinophils - We come in peace (or do we): Conspiracy, string and other theories from a consummate eosinophilophile</td>
<td>Steven Ackerman (USA)</td>
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**Coffee Break** - 10:00 to 10:30

**Eosinophils I** - 10:30 to 12:00

Chair Leo Koenderman (Netherlands)

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<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
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<tr>
<td>10:30</td>
<td>Eosinophil receptors, receptor-mediated inhibition</td>
<td>Ariel Munitz (Israel)</td>
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<td>10:50</td>
<td>Eosinophils regulate dendritic cell-induced Th2 polarization following allergen challenge in a mouse model of asthma</td>
<td>Elizabeth Jacobsen (USA)</td>
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<tr>
<td>11:30</td>
<td>Eosinophils and extracellular traps</td>
<td>Hans-Uwe Simon (Switzerland)</td>
</tr>
<tr>
<td>11:45</td>
<td>Impact of endocannabinoids on human eosinophils activation</td>
<td>Nicolas Flamand (Canada)</td>
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**Lunch** - 12:00 to 13:30 (on your own)
Eosinophils II - 13:30 to 15:00

Chair
Steven Ackerman (USA)

13:30 - 13:50
Eosinophils in the lung - Forever Young?
Madeleine Rådinger (Sweden)

13:50 - 14:10
Characterization of eosinophils in the zebrafish
David Traver (USA)

14:10 - 14:30
CD34 and eosinophils - beyond hematopoiesis
Kelly McNagny (Canada)

14:30 - 14:50
Identification of human eosinophil lineage-committed progenitors
Yasuo Mori (Japan)

14:50 - 15:00
Presentation of the 1st Gerald Gleich Award to Dr. Yasuo Mori
Gerald Gleich (USA)

Coffee - 15:00 to 15:30

Eosinophils III - 15:30 to 17:15

Chair
Paige Lacy (Canada)

15:30 - 15:50
Role of IL-25 in intestinal allergic inflammation
Yui-Hsi Wang (USA)

15:50 - 16:10
Eosinophil granule-derived cytokines: Mechanisms of secretion
Lisa Spencer (USA)

16:10 - 16:30
Signal transduction pathways involved in regulation of eosinophil differentiation
Miranda Buitenhuis (Netherlands)

16:30 - 16:45
Mechanisms of eosinophil accumulation in allergic diseases
Nives Zimmermann (USA)

16:45 - 17:00
What we have learned from ex vivo differentiated bone marrow eosinophils
Kimberly Dyer (USA)

17:00 - 17:15
Abstract Speaker
N-glycans and eosinophil trafficking
P. Sriramarao (USA)
## Thursday, June 23

### Breakfast (on your own)

**Eosinophils IV - 08:00 to 09:15**

**Chair**
David Abraham (USA)

- **08:00 - 08:20**
  - Ribonuclease-sensing by dendritic cells promotes Th2 responses
    Dragana Jankovic (USA)

- **08:20 - 08:40**
  - A TRAIL-regulated E3 ubiquitin ligase links allergen and rhinovirus exposure to asthma via targeting a protein phosphatase
    Joerg Mattes (Australia)

- **08:40 - 09:00**
  - Eosinophils regulate local immunity during parasitic nematode infection
    Judith Appleton (USA)

- **09:00 - 09:15**
  - Abstract speaker
    Eosinophils regulate local immunity during muscle infection by Trichinella spiralis
    Nebiat Gebreselassie (USA)

### Eosinophils in Health and Disease I - 09:20 to 10:00

**Chair**
Amy Klion (USA)

- **09:20 - 09:40**
  - Diagnosis and treatment of lymphocytic variant hypereosinophilic syndrome: state of the art and perspectives in 2011
    Florence Roufosse (Belgium)

- **09:40 - 10:00**
  - New classification of eosinophil hematopoietic disorders: report from Vienna meeting
    Peter Weller (USA)

### Coffee Break - 10:00 to 10:30

### Eosinophils in Health and Disease II - 10:30 to 11:55

**Chair**
Marc Rothenberg (USA)

- **10:30 - 10:45**
  - Gastrointestinal eosinophils: friend or foe?
    Glenn Furuta (USA)

- **10:45 - 11:00**
  - Monocyte/macrophage-CCL11-eosinophil axis in inflammatory bowel diseases
    Simon Hogan (USA)

- **11:00 - 11:15**
  - Mouse models of eosinophilic esophagitis
    Carine Blanchard (USA)

- **11:15 - 11:30**
  - Induction and activation of invariant natural killer T cells is critical in the initiation and progression of eosinophilic esophagitis
    Anil Mishra (USA)

- **11:30 - 11:55**
  - Genomic and MicroRNA analysis of Eosinophilic Esophagitis
    Marc Rothenberg (USA)

### Lunch - 12:00 to 13:30 (on your own)
Eosinophils in Health and Disease II (continued) - 13:30 to 13:55

Chair
Marc Rothenberg (USA)

13:30 - 13:50
Remodeling, eosinophils, and eosinophilic esophagitis
Seema Aceves (USA)

13:50 - 14:10
Medical treatment of eosinophilic esophagitis: New insights
Alex Straumann (Switzerland)

14:10 - 14:25
Abstract speaker
Hierarchical IL-5 expression defines a subpopulation of highly-differentiated “pro-eosinophilic” human Th2 cells
Calman Prussin (USA)

14:25 - 14:40
Abstract speaker
Role of IL-33 and eosinophils in intestinal inflammation
Joanne Masterson (USA)

14:40 - 14:55
Abstract speaker
The role of the small GTPase Rho H in eosinophil development and eosinophilic disorders
Christina Stoeckle (Switzerland)

Coffee Break - 15:00 to 15:30

IES Business Meeting (All are Welcome) – 15:30 -16:15

Eosinophils in Health and Disease III - 16:30 to 17:30

Chair
Kristin Leiferman (USA)

16:30 - 16:50
Eosinophils and dermatologic diseases
Dagmar Simon (Switzerland)

16:50 - 17:10
Eosinophils support the long term survival of plasma cells in the murine bone marrow
Claudia Berek (Germany)

17:10 - 17:30
Identification of innate IL-5 producing cells that promote eosinophil recruitment and contribute to antitumor immunity
Kiyoshi Takatsu (Japan)

Poster Preview - Highlights from the Podium - 17:30 to 18:00

Chair
Helene Rosenberg (USA)
SCIENTIFIC PROGRAM

FRIDAY, JUNE 24

Breakfast (on your own)

Eosinophils in Health and Disease IV - 08:00 to 09:50

Chair
A. Barry Kay (United Kingdom)

08:00 - 08:20
Asthma and eosinophils: can some controversies be overcome?
Frederick Hargreave (Canada)

08:20 - 08:40
Eosinophils and asthma; A link to exacerbations and remodeling
William Busse (USA)

08:40 - 09:00
Regulation of eosinophil progenitors in allergic asthma
Gail Gauvreau (Canada)

09:00 - 09:20
Human studies of eosinophils and allergic tissue reactions
A. Barry Kay (United Kingdom)

09:20 - 09:35
Abstract speaker
Inhibition of eosinophilic inflammation by supplementation with 5-hydroxytryptophan, a serotonin precursor
Joan Cook-Mills (USA)

Coffee and Poster Viewing - 9:35 to 10:25

Eosinophils in Health and Disease V - 10:25 to 12:00

Chair
Michael Minnicozzi (USA)

10:25 - 10:45
New insights into the roles of eosinophils in chronic rhinosinusitis
Robert Schleimer (USA)

10:45 - 11:05
Airway exposure to fungal antigens induces innate eosinophilia mediated by a novel Th2-type lymphoid cell subset
Hirohito Kita (USA)

11:05 - 11:25
A tale of two cells: Interactions between nerves and inflammatory cells in the lungs
Allison Fryer (USA)

11:25 - 11:45
Viruses, nerves, and eosinophils in asthma
David Jacoby (USA)

11:45 - 12:00
Noninvasive assessment of atopic diseases: Metabolomic profiling of urine using nuclear magnetic resonance (NMR) analysis
Darryl Adamko (Canada)

Lunch - 12:00 to 13:30 (on your own)
### Eosinophils and Therapy I - 13:30 to 15:00

**Chair**
Alex Straumann (Switzerland)

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<tr>
<td>13:30 - 13:50</td>
<td>Antagonists of IL-5 in asthma</td>
<td>Andy Wardlaw (United Kingdom)</td>
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<tr>
<td>13:50 - 14:10</td>
<td>Benralizumab, a humanized anti-interleukin 5 receptor-alpha monoclonal antibody, with enhanced antibody-dependent cell-mediated cytotoxicity function, in development for the treatment of asthma</td>
<td>Alison Humbles (USA)</td>
</tr>
<tr>
<td>14:10 - 14:30</td>
<td>Targeting eosinophil-specific siglecs</td>
<td>Bruce Bochner (USA)</td>
</tr>
<tr>
<td>14:30 - 14:45</td>
<td>Abstract speaker</td>
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<tr>
<td>14:45 - 15:00</td>
<td>The generation and characterization of inducible eosinophil-less transgenic mice</td>
<td>Lian Willetts (USA)</td>
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**Coffee and Poster Viewing** - 15:00 to 15:30

### Ehrlich Award Presentation and Lecture - 15:30 to 16:45

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<td>15:30 - 15:45</td>
<td>Introduction</td>
<td>Redwan Moqbel (Canada)</td>
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<td>15:45 - 16:30</td>
<td>Lecture: The attraction of eosinophiles</td>
<td>Timothy Williams (United Kingdom)</td>
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<td>16:30 - 16:45</td>
<td>Award Presentation</td>
<td>Redwan Moqbel (Canada)</td>
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**Poster Viewing** - 16:45 to 18:30

**Evening Activity** - 19:00 to 21:00
SCIENTIFIC PROGRAM

**Breakfast (on your own)**

**Personal Visions and Individual Directions - 08:00 to 10:00**

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<tr>
<td>08:00</td>
<td><strong>Eosinophils - Long a foot, but not effete</strong> Peter Weller (USA)</td>
</tr>
<tr>
<td>08:30</td>
<td><strong>My endless love story with eosinophils</strong> Monique Capron (France)</td>
</tr>
<tr>
<td>09:00</td>
<td><strong>From postdoc-to-fullstop</strong> Paul Foster (Australia)</td>
</tr>
<tr>
<td>09:30</td>
<td><strong>From parasites to allergy: Destiny strikes</strong> Francesca Levi-Schaffer (Israel)</td>
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</table>

**Coffee and Poster Viewing - 10:00 to 10:30**

**Personal Visions and Individual Directions (Continued) - 10:30 to 11:30**

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<tr>
<th>Time</th>
<th>Session</th>
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<tr>
<td>10:30</td>
<td><strong>The eosinophil as a teacher: “Basic” lessons and insights!</strong> Redwan Moqbel (Canada)</td>
</tr>
<tr>
<td>11:00</td>
<td><strong>Eosinophil priming and activation: It takes two to tango</strong> Leo Koenderman (Netherlands)</td>
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**Concluding Remarks - 11:30 to 12:00**

James Lee (USA)
The Ehrlich Lectureship is awarded at the biennial Eosinophil symposia of the International Eosinophil Society, Inc. to an individual(s) who has made seminal scientific contributions to research on the eosinophil and related allergy/immunology fields in terms of eosinophil biochemistry, development, cellular, molecular, structural or immunobiology and/or the participation of the eosinophil in the pathogenesis of Eosinophil-associated allergic or parasitic diseases and hypereosinophilic syndromes.

Professor Timothy J. Williams, PhD, FMedSci, is Emeritus Professor /Asthma UK Professor of Applied Pharmacology in the National Heart and Lung Institute at Imperial College, London, UK. Dr. Williams has served as a teacher, scholar, mentor, and leading researcher in the field of inflammation biology. He is a world-renowned expert in leukocyte biology, mechanisms of leukocyte recruitment, and pharmacologic interventions directed toward treatments of asthma and respiratory disease. His research reports have been cited prominently, and he has been recognized by numerous invited lectureships and awards, notably the Pfizer Prize (1984) and the Gaddum Memorial Prize (2000). We are delighted that he will be delivering the Paul Ehrlich Lecture at the 7th International Eosinophil Symposium, and receiving the Ehrlich Prize. This prize is awarded in recognition of Dr. Williams’ discovery of eotaxin, a molecule with unique eosinophil chemoattractant properties, and likewise for his profound contributions to the understanding of eosinophil trafficking, eosinophil-mediated inflammation, and the role of eosinophils in health and disease.

Recipient of the 2011 Ehrlich Lectureship:

Timothy J. Williams, PhD, FMedSci

For the discovery of Eotaxin and outstanding contributions to the understanding of eosinophil trafficking, eosinophil mediated inflammation and the role of eosinophils in health and disease
GLEICH AWARD

Dr. Yasuo Mori will receive the first Gerald Gleich prize. This prize will be awarded for the first time at Eosinophils 2011: Forever Young, and was created to recognize the individuals who have published high impact findings during the intervals since the preceding meeting. This award was named in honor of our esteemed colleague, Dr. Gerald Gleich, who has devoted his career to the exploration of the eosinophilic leukocyte and to the elucidation of its role in health and disease.

Dr. Mori was selected for this honor based on his first-authored publication “Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor” J Exp Med. 2009 Jan 16;206(1):183-93 which was from the laboratory of Dr. Koichi Akachi at Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan. In this work, Dr. Mori and Dr. Akachi defined an antigen profile for an unique eosinophil progenitor cell in human bone marrow and recognized its potential as a therapeutic target. We are delighted that Dr. Mori will be joining us at this meeting and receiving this award.

Recipient of the 2011 Gleich Award
Dr. Steven J. Ackerman, PhD, is the first recipient of the newly-established International Eosinophil Society Service Award. Dr. Ackerman is a graduate of McGill University, Montréal, Canada, and is currently Professor of Biochemistry and Molecular Genetics and Professor of Medicine at University of Illinois at Chicago. Dr. Ackerman, who is immediate past-president of the IES, has worked tirelessly on behalf of this organization. Among his activities, Dr. Ackerman organized the Third Eosinophil Symposium in Snowmass, Colorado, and co-organized and participated in fundraising activities for all of the meetings since then, including the organization, preparation, and submission of the NIH R13 conference grants. He served as Treasurer for the IES from 2003 until 2007, and both President and Treasurer from 2007 until 2009. During this time, he provided structure and financial stability to the fledgling organization. This prize is awarded to Dr. Ackerman in recognition of and gratitude for the dedicated service he has provided to the International Eosinophil Society and to the larger community of eosinophil scientists worldwide.

Recipient of the 2011 Service Award
"WHAT LIES BEFORE OUR EYES MAY BE LIES: EOSINOPHIL DEGRANULATION---IN THE ABSENCE OF INTACT EOSINOPHILS"

Gerald J. Gleich, MD
Departments of Dermatology and Medicine
University of Utah Health Sciences Center
Salt Lake City, UT 84132

The eosinophil may be the most unique cell in the body with its distinctive granules. Once the eosinophil gains access to a site of inflammation, it often releases granule proteins, and these can be detected using specific antibodies. Typically, biopsies specimens from eosinophil-associated disease tissues show intact eosinophils along with granule protein deposition. In some cases, however, a biopsy from a site of inflammation is devoid of intact eosinophils or reveals scant infiltration whereas localization of eosinophil granule proteins shows striking deposition. In this presentation, I will show evidence that biopsies from patients with cutaneous eosinophil-associated disease and with cardiac disease demonstrate this phenomenon. A recent patient well exemplifies the importance of searching for evidence of eosinophil granule protein deposition. This 70-year-old male with marked peripheral blood eosinophilia, after an extended asymptomatic time, suffered several small strokes. Because of his peripheral blood eosinophilia, evidence of endomyocardial disease was sought utilizing echocardiography; four echocardiographs, including one transesophageal echocardiogram, failed to show evidence of cardiac abnormality. An endomyocardial biopsy was performed which showed minimal inflammation and essentially no eosinophil infiltration. However, when stained with antibodies to the eosinophil granule major basic protein-1, the same biopsy showed striking deposition of this granule protein. Once the implications of this finding were recognized, the patient was treated with peginterferon alfa and has made a steady recovery. This case highlights the importance of recognizing that eosinophil involvement in disease is not always signaled by the presence of intact cells, but may be recognized only by granule protein deposition.
OF MICE AND MEN - ECP MAKES THE DIFFERENCE?
Per Venge
Department of Medical Sciences, University of Uppsala, Uppsala, Sweden

Background: Eosinophil Cationic Protein (ECP) or RNase 3 is the result of a gene duplication that occurred millions of years ago. The gene and its product are only found in human and primate eosinophils. One consequence of the gene duplication was the loss of RNase activity, but the gain of cytotoxic properties. The other part of the duplicated gene i.e. Eosinophil Protein-X/Eosinophil Derived Neurotoxin (EPX/EDN) or RNase 2, retained its RNase activity. Also the EPX/EDN gene is unique to primates and humans, but homologues proteins with RNase activities are found in most mammals.

Objectives: To summarize our knowledge of the biology of ECP and the possible impact of ECP on diseases unique to the human species.

Results: ECP is a truly multifunctional protein with both cytotoxic and non-cytotoxic properties. In addition to a weak RNase activity, the non-cytotoxic activities include fibrogenic activities, interactions and modifications of mast cell and B and T-lymphocyte activities etc.(reviewed in J. Byström et al, 2011). ECP is stored in the eosinophil granules in various glycosylated forms, which have little or no cytotoxic activity. Upon release the molecule is partly deglycosylated and acquires cytotoxic activity. One polymorphism in the coding part of exon 2 results in the change of the amino acid arginine at position 97 to threonin and consequently loss of cytotoxic activity (without affecting the RNase activity) due to the formation of an additional glycosylation site. Other polymorphisms in the non-coding parts of the gene i.e. in the intron and in the 5’UTR sequence have impact on the production. Associations of ECP to human disease are suggested by findings in allergic disease such as asthma and rhinitis, but also in fibrotic disease. Thus, highly elevated ECP levels were found in most body fluids in these diseases with correlations to signs and symptoms and outcome of the diseases. Such data are obviously difficult to interpret since they may rather reflect the overall impact of eosinophils and not the sole impact of ECP. However, allergic diseases such as asthma are unique to humans although allergic reactions and asthma-like conditions can be induced in experimental animals. An alternative to find out about the unique role of ECP in humans is therefore to answer the question whether the genetics of ECP, with alterations in functions and productions, might relate to these unique human diseases. Such studies have shown intriguing associations of ECP gene polymorphisms to the development of allergic symptoms and the development of fibrotic lesions, but also to susceptibility to parasitic infections.

Conclusions: ECP is a unique gene product of primate and human eosinophils. The associations of ECP to allergic and fibrotic disease that are unique to humans suggest that the counteraction of the activities of ECP might be an alternative way to treat such diseases. Our findings emphasize the difference between mice and men and that farfetched extrapolations from animal experiments to humans about the role of eosinophils in human disease should be made with caution.

THE EOSINOPHIL AMBASSADORS:
EOSINOPHILS – WE COME IN PEACE (OR DO WE): CONSPIRACY, STRING AND OTHER THEORIES FROM A CONSUMMATE EOSINOPHILE

Steven J. Ackerman, PhD
Professor, Department of Biochemistry and Molecular Genetics, and Sections of Hematology-Oncology and Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, College of Medicine, University of Illinois at Chicago, IL 60607 USA

Background: The past 50+ years have seen many novel and interesting theories come and go regarding eosinophil biology and their roles in host immune defense against multicellular parasites, anti-inflammatory and pro-inflammatory activities in the pathogenesis of asthma and other allergic diseases, regulation of tissue microenvironments, induction of tissue remodeling and fibrosis, antigen presentation, and most recently links between innate and adaptive immunity in the generation of T-cell responses (to quote our IES president, “whose your daddy now T cell”, J Lee, 2009).

Objective: Since as an eosinophil ambassador I now have diplomatic immunity, my objective is to take this opportunity to discuss or debunk some of these great (or not so great) ideas and theories in eosinophil biology, including recent links to string theory and a universal “eosinophil theory of everything”.

Methods: In promulgating my esoteric cogitations and articulating my superficial sentimentalities and amicable philosophical or psychological observations on “great ideas in eosinophil biology”, I plan to beware of platitudinous ponderosity, letting my conversational communication possess a compacted conciseness; a certain clarified comprehensibility, coalescent cogency and concatenated consistency. I will above all attempt to eschew obfuscation and all conglomerations of flatulent garrulity, jejune babblement and asinine affectations, allowing extemporaneous expatiations that have intelligibility and voracious vivacity without rodomontade or thrasonical bombast. I will therefore sedulously avoid all polysyllabic profundity, pompous prolificacy and vain vapid verbosity.

Results: In a nutshell, with only a scant 15 minutes to speak, I plan to be brief, not use big words, not repeat myself and above all, allow no time for rebuttal!

Conclusions: (1) There are still more questions than answers – good for the field and grant applications! (2) Eosinophils are our friends – they come in peace – or do they?

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EOSINOPHIL RECEPTORS AND RECEPTOR MEDIATED INHIBITION

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An intricate network of activation and inhibitory signals tightly regulates the cellular responses of various immune cells including eosinophils. To date, multiple activation receptors have been described on eosinophils such as cytokine and chemokine receptors, Fc receptors, and complement receptors. These receptors mediate eosinophil chemotaxis, adhesion, mediator release and survival. In contrast to these activation pathways, an opposing and suppressive receptor system has evolved that has been collectively termed “inhibitory receptors”. The prototype immune inhibitory receptor is identified by a consensus amino acid sequence, the immunoreceptor tyrosine-based inhibitory motif (ITIM), which is present in the cytoplasmic domain of these receptors. Upon receptor:ligand engagement, the ITIM(s) undergo tyrosine phosphorylation and recruit cytoplasmic phosphatases such as the SH2-containing phosphatases-1 (SHP-1) and -2. Inhibitory receptors are thus capable to suppress various cellular responses. Indeed, gene targeted mice lacking inhibitory receptors are more susceptible to inflammation and autoimmunity. Surprisingly, and despite the key roles of inhibitory receptors in immune cell functions, their expression and roles in the regulation of eosinophil responses has received scant attention. Over the last couple of years we focused on the role of the inhibitory receptors paired immunoglobulin-like receptor B (PIR-B), CMRF-like molecule 1 and 8 (also termed CD300lf and CD300a, respectively) in eosinophil migration, survival and mediator release. Basic structure and mechanism of action of these inhibitory receptors will be outlined, as well as a summary of recent advances regarding the roles of such receptors in eosinophils. Finally, the concept of utilizing inhibitory receptors as pharmacological targets for eosinophil suppression will be discussed.
EOSINOPHILS REGULATE DENDRITIC CELL-INDUCED TH2 POLARIZATION FOLLOWING ALLERGEN CHALLENGE IN A MOUSE MODEL OF ASTHMA

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Rationale: Eosinophil recruitment and accumulation in the lungs of asthmatics is a hallmark feature of asthma. The causative relationship between eosinophils and induced Th2 pulmonary pathologies remains unclear. Our data suggest a novel role for eosinophils as immune modulators that drive Th2-mediated pulmonary pathologies through modulation of dendritic cell function leading to the suppression of Th1/Th17 immune responses.

Objective: To demonstrate that pulmonary and circulating eosinophils have distinct roles in activating and polarizing T cells in a mouse model of allergic pulmonary inflammation.

Methods: Mice for these experiments are wild type, transgenic eosinophil-deficient (PHIL), or IL-5 knockout (IL-5-/-) mice on a C57BL/6J background. PHIL mice fail to induce allergen-induced pulmonary pathologies and IL-5-/- mice elicit only a nominal eosinophil response in the lungs following allergen provocation (~92% reduction relative to allergen exposed wild type animals). Purified blood eosinophils were either adoptively transferred directly into the lung (i.e., intratracheal instillation (i.t.)) or indirectly via peripheral circulation (i.e., intraperitoneal (i.p.)) or both into PHIL or IL-5-/- mice at the time of allergen challenge using an established acute ovalbumin (OVA) sensitization/challenge protocol. Additionally, MHCII deficient eosinophils and eosinophils treated with Th2 cytokines were transferred to determine the eosinophil migration, T cell activation, and modulation of dendritic cells. Finally, ovalbumin (OVA)-pulsed myeloid dendritic cells were adoptively transferred into wild type or PHIL mice at the time of allergen challenge using an established acute OVA protocol. Allergen-induced pulmonary changes were assessed as changes in lung and lung draining lymph node (LDLN) cells, activation/polarization of T cells, and induced lung histopathologies.

Results: We demonstrate that a threshold level of eosinophils must migrate to the lung draining lymph node to promote myeloid dendritic cell recruitment and activation of antigen-specific memory T cells. Eosinophils perform this function either through direct migration of blood eosinophils to the LDLN from the intraperitoneal cavity or after activation with Th2 cytokines and introduction into the lung. Expression of MHC II or CCR7 on eosinophils is not necessary for proliferation of T cells in the LDLN. These lymphatic eosinophils are necessary to suppress Th1/Th17 neutrophilic polarized immune responses initiated by OVA-pulsed dendritic cells during allergen challenge.

Conclusion: These results imply an important function for eosinophils in modulating the balance between Th2 and Th1/Th17 pathways in the development of allergic respiratory inflammation.

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EOSINOPHILS AND EXTRACELLULAR TRAPS

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Although eosinophils are considered as being useful in defense mechanisms against parasites, their exact function(s) in innate immunity remains unclear. We have recently obtained evidence for a novel eosinophil-mediated defense mechanism that seems to play a role in the gastrointestinal immune system. We show that activated eosinophils are able to release mitochondrial DNA. Strikingly, the process of DNA release occurs with high speed in a catapult-like manner in less than 1 second. In the extracellular space, the mitochondrial DNA and granule proteins form extracellular structures able to bind and kill bacteria. Therefore, we called these DNA-containing structures eosinophil extracellular traps (EETs). Meanwhile, we identified EETs in multiple infectious, autoimmune and allergic diseases. Although the role of the EETs remains unclear and further work is required for better understanding of this phenomenon associated with eosinophilic inflammation, current observations suggest that EETs could serve as an important innate immunity mechanism protecting skin and mucosa from invasion by infectious factors.
**EOSINOPHILS IN THE LUNG - FOREVER YOUNG?**

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**Background:** Allergic airway inflammation in asthma is dominated by eosinophils, which develop from CD34+ hematopoietic progenitor cells within the bone marrow. Emerging evidence suggests that CD34+ progenitor cells migrate from bone marrow to sites of allergic inflammation where they can undergo further proliferation and final maturation, potentially augmenting the degree of tissue inflammation. To date, however, it is not clear which chemotactic factors induce the traffic of CD34+ cells to the airways during the allergic response or which factors affect the in situ proliferation and survival of these cells. However, the eotaxin receptor, CCR3 has been shown to be expressed on CD34+ bone marrow cells and asthmatics with late responses to allergen have increased numbers of bone marrow CCR3+/CD34+ cells 24 hours after allergen challenge.

**Objectives:** We hypothesize that CD34+/CCR3+ cells are increased in the airways after allergen exposure. We further hypothesize that these cells, in addition to the classical CD34+/IL-5Receptor alpha+ (IL-5Ralpha+) eosinophil progenitor cells have a proliferative capacity and undergo in situ proliferation in response to allergen.

**Methods:** A well-characterized mouse model of allergen-induced airway inflammation was used to determine the role of CCR3 receptor-ligand interaction in the migration and function of CD34+ cells. To further investigate whether the CD34+/CCR3+ airway cells have a hematopoietic stem cell phenotype, the expression of Stem cell antigen-1 (Sca-1) or IL-5 Receptor alpha on these cells was also assessed. A thymidine analogue (5-bromo-2´deoxyuridine; BrdU) was used to label newly produced in vivo proliferating lung cells and newly produced proliferating CD34+/CCR3+, Sca-1+/CCR3+ and IL-5Ralpha+ cells was enumerated by flow cytometry. In vitro proliferation of lung CD34+ cells was evaluated by semi solid cultures, evaluating colony formation (CFU). In addition, eotaxin-2 was delivered to the airways of IL-5 transgenic mice and in vitro migration of CD34+/CCR3+ bone marrow and blood cells in response to eotaxin-1 and eotaxin-2 was assessed to further delineate the role of these chemokines in CD34+/CCR3+ cell mobilization. Moreover, the specific role of these progenitor populations in pulmonary allergen-mediated inflammatory responses was highlighted in vivo by selective depletion with a rat anti-mouse CCR3 monoclonal antibody.

**Results:** Allergen exposure significantly increased bone marrow, blood and airway CD34+/CCR3+ cells as well as airway CD34+/CCR3+ /Sca-1+ and CD34+/CD45+/IL-5Ralpha+ cells. A portion of the newly produced CD34+/CCR3+, Sca-1+/CCR3+ and IL-5Ralpha+ lung cells showed a significant proliferative capacity in response to allergen when compared with saline treated animals. Significantly, IL-5 and eotaxin-2 each alone stimulated in vitro CFUs of lung CD34+ cells. Moreover, delivery of eotaxin-2 to the airways of IL-5 transgenic mice resulted in a substantial increase of CD34+ cells in the airways. Finally, systemic treatment with a depleting anti-CCR3 antibody abolished both CD34+ and Sca-1+ cells in airways to levels similar to control animals.

**Conclusions:** These data suggest that the CCR3/eotaxin pathway is involved in the regulation of allergen-driven in situ hematopoiesis and the accumulation/mobilization of eosinophil-lineage committed progenitor cells in the lung. Thus, targeting both IL-5 and CCR3-mediated signaling pathways might be needed in order to control the inflammation associated with allergen induced asthma.

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CHARACTERIZATION OF EOSINOPHILS IN THE ZEBRAFISH

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Eosinophils are multifunctional granulocytes implicated in numerous aspects of immunity and disease. Despite their unmistakable presence during chronic inflammation, the precise functions of eosinophils remain enigmatic. Developing alternative models to study eosinophil biology may yield new information regarding their functions. Eosinophils have been observed in zebrafish but have not been thoroughly characterized. We utilized a gata2:eGFP transgenic animal to enable prospective isolation and characterization of zebrafish eosinophils. We demonstrate that all gata2hi cells in adult zebrafish are eosinophils, and that their light-scatter characteristics are distinct from other leukocyte subsets. Although eosinophils are rare in most organs, they are readily isolated from whole kidney marrow (WKM) and abundant within the peritoneal cavity. Eosinophils within WKM displayed immature morphological characteristics, in contrast to mature, polymorphonuclear morphologies evident within the peritoneum. Gene expression analyses of purified gata2hi cells demonstrated that zebrafish eosinophils express many genes previously shown to be important for eosinophil differentiation, activation, and degranulation in mammals. Furthermore, gata2hi cells degranulate in response to helminth extract as determined by colorimetric and ultrastructural analyses. Together, these observations suggest that the zebrafish will provide a useful model to better understand the roles of eosinophils in health and disease.
CD34 AND EOSINOPHILS: BEYOND HEMATOPOIESIS

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Background: Cell surface sialomucins are a large and diverse family of plasma membrane proteins characterized by an extensively O-linked glycosylated extracellular domain. CD34 is the founding member of one sialomucin subfamily and is best known as a marker of hematopoietic progenitor/stem cells. Intriguingly, we find that it is also a selective marker of eosinophils, mast cells and dendritic cells (DCs).

Objectives: We have used CD34-deficient mice as a tool to evaluate its function on these inflammatory cells in a variety of disease models.

Methods: Wildtype (Wt) and Cd34-/- mice were evaluated in standard models of: 1) Ova-induced asthma [1], 2) DSS-induced colitis [2] and 3) a model of bacterial antigen-induced lung hypersensitivity pneumonitis (HP). In each of these disease models, the frequency and types of tissue infiltrating inflammatory cells was evaluated as well as the degree of pathology via histology. In addition, the ability of inflammatory cells to migrate and in response to chemokines was evaluated by standard transwell assays in vitro.

Results: We find that Cd34-/- mice are remarkably resistant in all 3 models of mucosal inflammatory disease. This protection is not due to an impaired ability to produce inflammatory cell precursors, as all hematopoietic subsets are present in normal frequencies in the bone marrow. However, in Ova-induced asthma (a Th2-type inflammatory disease) we find a clear reduction in the frequency of eosinophils recruited to the lung parenchyma and bronchoalveolar lavage fluid and instead find an accumulation of eosinophils in the peripheral blood [1]. Similarly, in DSS-induced colitis, we find dampened eosinophil recruitment to the gut mucosa [2]. In vitro, CD34-deficient eosinophils exhibit an impaired ability to chemotax effectively to the CCR3-ligand, eotaxin 1.

HP, in contrast to asthma, is an eosinophil- and mast cell-independent Th1/Th17-biased inflammatory disease. In this model, we find that Cd34-/- DCs exhibit defective trafficking to the lung alveoli and the draining lymph nodes. Correspondingly, these mice failed to develop lung inflammatory infiltrates typical of secondary immune responses and instead mimic primary responses. In vitro, Cd34-/- DCs exhibit an impaired ability to chemotax to CCL19 over a range of concentrations but exhibit a Wt ability to chemotax to CXCL12. Thus, our data suggest that rather than playing a global role in regulating chemotaxis, CD34 plays a much more selective role in enhancing the fidelity of chemotaxis to a subset of chemokines.

Conclusions: It has long been thought that cell surface sialomucins play a biophysical role in either enhancing or reducing adhesion based on their degree and type of glycosylation. Our data, in contrast, suggest that CD34-type sialomucins may play a much more selective role in enhancing the fidelity of chemokine-dependent migration. This may, in fact, represent a new general paradigm for the larger family of cell surface sialomucins.

References:


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IDENTIFICATION OF HUMAN EOSINOPHIL LINEAGE-COMMITTED PROGENITORS

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Background: Eosinophils originate from hematopoietic stem cells in the bone marrow, but their developmental mechanism and its pathway remains unclear. Recent advances in the multi-color FACS system have enabled us to isolate the murine eosinophil lineage-committed progenitors (mEoPs) [1].

Objectives: We aimed to identify the human (h) counterpart of mEoPs (hEoPs), to delineate the developmental pathway of hEoPs, and to evaluate whether hEoP population are involved in the pathogenesis of eosinophil mediated disorders.

Methods: Blood and bone marrow samples were obtained from patients with eosinophilia and from normal volunteers. Multicolor FACS analyses and sorting were performed by FACSaria cell-sorting system. Hematopoietic stem cells (hHSCs) and myeloid progenitors (common myeloid progenitors; hCMPs, granulocyte/macrophage progenitors; hGMPs, and megakaryocyte/erythrocyte progenitors; hMEPs) were isolated as previously described [2]. We evaluated the lineage readout by liquid cultures and methylcellulose colony assays with supplementation of cytokine cocktail. For gene expression analysis, total RNA was extracted from purified progenitor populations, cultured cells, or mature blood granulocytes. The conventional RT-PCR and the qPCR assays were performed with the GeneAmp 9700 PCR System and the PRISM 7500 Fast Real-Time PCR System, respectively (Applied Biosystems).

Results: IL-5Ra was expressed on blood mature eosinophils as well as on basophils at high levels in humans. Liquid cultures and colony assays revealed eosinophilic progenitors developed from hHSCs and hCMPs but not from hGMPs and hMEPs. In addition, IL-5Rα-expressing progenitors were detected only in hCMP population. These IL-5Rα+ fraction within hCMP population gave rise only to pure eosinophil colonies with ~20% of plating efficiency. In contrast, the IL-5Rα− fraction of hCMPs generated a variety of myeloid colonies including rare eosinophil-containing colonies. These observations collectively suggested that the IL-5Rα+ fraction of original hCMP has committed to the eosinophil lineage. Gene expression analyses revealed that the expression pattern of major transcription factors was well conserved between mice and human at least the EoP stage. In brief, up-regulation of GATAs but not FOG-1 with maintained expression of PU.1 and C/EBPα instructed the eosinophil-lineage differentiation. The hEoP population significantly expanded in patients with eosinophilia compared to that in healthy individuals (7.44% vs. 2.38% of bone marrow CD34+ cells, p < .05).

Conclusions: We successfully identified hEoPs in the bone marrow as the IL-5Rα+CD34+CD38+IL-3Rα+CD45RA− population, which develop downstream of hCMPs independent of hGMPs. The phenotypic definition of original hCMPs should be revised as IL-5Rα−CD34+CD38+IL-3Rα+CD45RA−. Eosinophil lineage commitment was regulated by several transcription factors. hEoP population played a critical role in the expansion of eosinophils and could be a therapeutic target for eosinophil-mediated disorders.

References:

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ROLE OF IL-25 IN INTESTINAL ALLERGIC INFLAMMATION

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Background: Food allergen-induced diarrhea is a hypersensitivity reaction that involves IgE-mediated humoral immune responses and T\(_h\)2 cell mediated cellular inflammatory responses. IL-25 (IL-17E), a distinct IL-17 inflammatory cytokine member, induces elevated T\(_h\)2 cytokine production, in particular IL-5, which drives eosinophilia and pathological changes during allergic inflammation.

Objectives: We hypothesize that food allergens induce elevated intestinal IL-25 that potentiates allergic sensitization and augments T\(_h\)2 cytokine production by local T\(_h\)2 memory/effector cells, leading to the exacerbation of gastrointestinal (GI) allergic inflammation.

Methods: Wild type or intestinal specific IL-25Tg (iFABP-IL-25) mice were sensitized with OVA plus alum twice before six times of repetitive OVA challenge intragastrically. Intestinal tissues were harvested one day after the last challenges. Total RNA was isolated from mouse intestinal epithelial cells or total tissues and expression levels of examined genes normalized to control gapdh gene was determined using real time PCR method. Histological analysis of the duodenum from IL-25Tg or wild type BALB/c mice was performed by staining with hematoxylin and eosin (H&E) or MBP (myelin basic protein).

Results: In a mouse model of OVA-induced allergic diarrhea, intestinal epithelial IL-25 gene expression was rapidly induced after only two times of OVA challenge intragastrically, despite that the occurrence of diarrhea requires at least six times of challenges. IL-25 transcript expression was primarily elevated in the duodenum, but not other sections of intestine. In addition to IL-25, gene expression of TH2 cytokine IL-5, IL-13, and in particular, IL-9, and chemokine eotaxin-1 were induced at the early phase of intestinal allergic responses. Notably, TH2 cells in laminar propria expressed high level of surface IL-25R and produced large amount of TH2 cytokines IL-5, IL-13 and IL-9. Transgenic mice overexpressing intestinal IL-25 exhibit goblet hyperplasia and increased infiltration of eosinophils, resulting in their susceptibility to OVA-induced rectal temperature changes and allergic diarrhea.

Conclusion: Induction of IL-25 expression in the intestinal duodenum precedes the onset of OVA-induced intestinal anaphylaxis. Elevated intestinal IL-25 production potentiates allergic sensitization and augments TH2 cytokine production, in particular IL-9 by local TH2 cells, leading to intestinal eosinophilia and the exacerbation of effector phase of GI allergic inflammation.

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EOSINOPHIL GRANULE-DERIVED CYTOKINES: MECHANISMS OF SECRETION

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One essential mechanism by which eosinophils accomplish their varied functions in health and disease is through the rapid secretion of cytokines, chemokines and growth factors. Eosinophil-derived cytokines engage tissue cells, nerve cells, and innate and adaptive immune cells, and are implicated in diverse processes, including immunomodulation, cellular recruitment, and tissue repair and remodeling associated with a variety of allergic inflammatory diseases.

Distinguishing eosinophils from many other immune cells, eosinophil cytokine secretion can be elicited within minutes of agonist stimulation, primarily through a mechanism of piecemeal degranulation that draws upon preformed reserves of cytokine proteins stored within intracellular granules and transport vesicles. Using a complementary approach combining microscopy with molecular and biochemical assays, we have elucidated the relative preformed cytokine potential of blood eosinophils from healthy individuals, and visualized the dynamic processes of mediator packaging and vesicle formation and release from eosinophil intracellular granules. Moreover, we identified a novel mechanism of cognate receptor-mediated trafficking by which selectivity of stimulus-induced cytokine release may be achieved.

In addition to piecemeal degranulation, eosinophils within tissues can follow a program of cytolytic degranulation, whereby intact intracellular granules are extruded from dying eosinophils and deposited in clusters within the surrounding tissue. Utilizing as a starting material cell-free human eosinophil granules recovered from either subcellular fractionations of eosinophil cavitates, or from the supernatants of eosinophils exposed to human serum-coated beads, we have found eosinophil granules to express functional chemokine and cytokine receptors, and to differentially secrete cytokines in response to activation of these receptors. Thus, cell-free eosinophil granules deposited within tissues may continue to function as secretory competent organelles.

Taken together, this work delineates the major mechanisms by which eosinophils secrete cytokines from preformed intragranular caches, enabling the many cytokine-dependent biological roles of eosinophils in health and diseases.

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INVITED SPEAKER ABSTRACTS

SIGNAL TRANSDUCTION PATHWAYS INVOLVED IN REGULATION OF EOSINOPHIL DIFFERENTIATION

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The development of all blood cells, including eosinophils, is a complex series of events regulated by cytokines at multiple levels, including proliferation, survival and differentiation. Cytokines bind to their cognate receptors and regulate the activity of intracellular signal transduction pathways leading to modulation of gene expression by regulation of transcription factor activity. In contrast to transcription factors, the role of signal transduction molecules in regulation of hematopoiesis has until recently remained undefined. Most of the currently known effectors that are involved in hematopoiesis, including PKB and p38MAPK, are regulated by cytokines in a similar manner. Although the activity of both PKB and p38MAPK is induced upon cytokine stimulation, these molecules appear to counteract each other during hematopoiesis. Whereas, for example, neutrophil differentiation is positively regulated by PKB, it is inhibited by p38MAPK. In contrast, both activation of p38 MAPK and/or inhibition of PKB activity induce eosinophil differentiation. Similarly, in contrast to activation of p38MAPK that results in inhibitory phosphorylation of C/EBPa on Ser21, activation of PKB induces dephosphorylation and activation of C/EBPa (Thr226/226). Ectopic expression of a non-phosphorylatable C/EBPa mutant (either T222/226A or S21A) in hematopoietic progenitors was sufficient to induce neutrophil development and to block eosinophil differentiation. Since IL-5 induces PKB phosphorylation, and inhibition of PKB activity appear to be essential for eosinophil maturation, it could be hypothesized that components of the hematopoietic stem cell niche inhibit PKB activity. Interestingly, bone marrow derived mesenchymal stromal cells appear to abrogate cytokine mediated induction of PKB phosphorylation in hematopoietic progenitors, suggesting that those cells might play an important role in eosinophil maturation.
MECHANISMS OF EOSINOPHIL ACCUMULATION IN ALLERGIC DISEASES

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Eosinophil accumulation is increased in allergic diseases, and this accumulation has been associated with disease pathogenesis. Accumulation can be regulated by multiple processes including recruitment of mature eosinophils, in situ differentiation from progenitors, egress, and cell death. Recent studies in our laboratory have focused on the role and mechanism of cell death in eosinophil accumulation in allergic disease.

Cell death appears to be an important regulator of eosinophil accumulation in disease as prolonged survival of eosinophils is a characteristic feature of asthma, and the number of apoptotic eosinophils is inversely correlated with clinical severity of asthma. While eosinophils encounter multiple survival and death-inducing signals in diseased tissue, the process of cell fate determination is poorly understood. Here, we will focus on two signaling pathways and discuss possible integration mechanisms in this process.

The microenvironment of the lung in asthma is acidic, yet the effect of acidity on inflammatory cells has not been well established. We recently demonstrated that acidity inhibits eosinophil apoptosis and increases cellular viability. Notably, acidity induced eosinophil cAMP production and enhanced cellular viability in an adenylyl cyclase-dependent manner. Furthermore, we identified G protein-coupled receptor 65 (GPR65) as the chief acid-sensing receptor expressed by eosinophils, as GPR65-deficient eosinophils were resistant to acid-induced eosinophil cAMP production and enhanced viability. Notably, GPR65(-/-) mice had attenuated airway eosinophilia and increased apoptosis in two distinct models of allergic airway disease. We conclude that eosinophil viability is increased in acidic microenvironments in a cAMP- and GPR65-dependent manner.

Recently, we and others identified the eosinophil-selective receptor Siglec-8, a member of the sialic acid-binding, immunoglobulin-like lectin family with cytoplasmic ITIM domains. Antibody engagement of Siglec-8 induces eosinophil cell death. In vivo targeting of its closest mouse counterpart, Siglec-F, results in eosinophil apoptosis, reduced eosinophilic inflammation, and decreased tissue remodeling in models of asthma and gastrointestinal complications of food allergy. Siglec-8 crosslinking leads to eosinophil cell death via reactive oxygen species and mitochondrial pathways. However, the membrane-proximal signal transduction events are not known. An initial signaling molecule screen following Siglec-8 crosslinking identified several signal transduction molecules, including Lyn, Crk, and Vav, whose phosphorylation and/or association were increased. Since Lyn has previously been identified to have a role in signaling of ITIM-bearing receptors and apoptosis, we focused on this member of the Src kinase family. Using two chemically distinct inhibitors, we demonstrated that a Src kinase family member is required for Siglec-8-induced apoptosis. Thus, our data identify a novel signaling paradigm for the Siglec family of receptors.

Paradoxically, the eosinophil survival factor IL-5 enhances Siglec-8-induced eosinophil cell death. Similarly, survival-inducing acidity enhanced Fas-induced eosinophil cell death. As the mechanisms of signal integration are still poorly understood, future studies should focus on understanding the mechanisms of signal integration in disease states and the effect of various survival factors and mediators on disease pathogenesis and outcomes.

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WHAT WE HAVE LEARNED FROM EX VIVO DIFFERENTIATED BONE MARROW EOSINOPHILS

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Among the difficulties encountered by eosinophil biologists is the fact that eosinophil-specific events represent a fraction of the ongoing hematopoietic activity in bone marrow at any given time, even under profound Th2 stimulation. We have developed an ex vivo culture system using SCF and FLt3L in combination with IL-5 to generate large numbers of eosinophils at high purity (>90%) from unselected mouse bone marrow progenitors [1]. This method has been used to generate eosinophils from both BALB/c and C57BL/6 strains of mice and a variety of gene-ablated mice [2]. The bone-marrow derived eosinophils (bmEos) look like eosinophils, and express immunoreactive major basic protein, Siglec F, IL-5 receptor alpha, and transcripts encoding mouse eosinophil peroxidase, CC chemokine receptor 3, the IL-3/IL-5/GM-CSF receptor common beta-chain (βc), and the transcription factor GATA-1. The bmEos are functionally competent, as they undergo chemotaxis toward mouse eotaxin-1 and produce characteristic cytokines, including interferon-γ, IL-4, MIP-1α and IL-6.

We and others have used bmEos generated ex vivo from this culture system to explore several important physiologic responses. Examination of bmEos generated from wild-type and platelet-activating factor receptor (PAFR) gene-deleted mice led to identification of both platelet activating factor (PAF) and lysoPAFAs secretagogues for mouse eosinophils [3] and provided evidence suggesting that degranulation via these mediators utilizes a non-classical PAF receptor. Furthermore, we found that mouse bmEos release cytokines in response to IL-6, but not in response to PAF or lysoPAF, suggesting piecemeal degranulation (PMD) as has been characterized for human eosinophils. In another study, Rankin and colleagues [4] differentiated bmEos from wild-type and IL-33 receptor subunit IL-1R4/ST2 gene-deleted progenitors, and showed that IL-33 stimulated the release of IL-13 from bmEos in an ST2-dependent manner. These results suggest a role for eosinophil-derived IL-13 in the development of IL-33-induced physiologic sequelae. In a third study, our group has explored direct infection of eosinophils with the rodent respiratory pathogen, pneumonia virus of mice (PVM [5]). PVM replicates in bmEos, and virions are released from infected cells, as are proinflammatory cytokines IP-10, IL-6, MCP-1 and MIP-1alpha. PVM replication is augmented in MyD88 gene-deleted bmEos, which is suppressed by exogenous IL-6.

Taken together, these findings highlight the utility of this method via which mouse eosinophils are obtained in sufficient numbers to perform biologically meaningful experiments.

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RIBONUCLEASE-SENSING BY DENDRITIC CELLS PROMOTES TH2 RESPONSES

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CD4+ T cells are now known to consist of three major subsets Th1, Th2 and Th17 that display distinct functions in both host defense and immunopathologic responses. For example, Th2 lymphocytes play an essential role in protection against helminth infections but also promote the pathogenesis of asthma and allergy. The delineation of the signals that trigger differentiation of these CD4 subpopulations is important for the development of interventions based on the selective induction or ablation of specific Th effectors. It is well documented that activation of dendritic cell (DC), through the engagement of pattern-recognition receptors (e.g. TLRs), is critical for microbial induced development of Th1 and Th17 cells. In contrast, the mechanism by which Th2 immune responses are triggered is unclear both in terms of the recognition events and signaling pathways involved.

Although helminth parasites are the most potent microbial stimulus for Th2 polarization, the molecular components of these pathogens responsible for Th2 induction are poorly defined and in particular those that act by conditioning DC function. Schistosoma mansoni eggs and their soluble products are perhaps the most widely studied helminth derived Th2 promoting agents. SEA is complex mixture of hundreds of proteins and glycoconjugates that include structural components of the miracidial embryo as well as its secretory products. In terms of single molecules, IPSE/alpha-1 and peroxiredoxin trigger IgE-dependent IL-4-secretion by basophils and induce alternatively activated macrophages, respectively, but neither are known to directly interact with DC. Recently, we purified and identified the component in schistosome eggs responsible for DC-dependent Th2 polarization and showed that it is a single 32 kD egg-secreted glycoprotein with ribonuclease activity, S. mansoni T2 ribonuclease/omega-1. We further demonstrate that purified omega-1 does not induce ‘classical’ activation of DC but instead triggers morphological/cytoskeletal changes in DC linked to decreased antigen-dependent conjugate formation with CD4+ T lymphocytes. The end result is decreased CD4+ T cell activation, a situation previously shown to favor Th2 polarization under conditions of low-dose antigen exposure.

Interestingly, ribonucleases secreted by eosinophils, have been also shown to promote Th2 responses. Moreover, several inhaled allergens in fungi and grass pollen have been characterized as ribonucleases. Therefore, we are currently testing the general hypothesis that the sensing by DC of ribonucleases is an important pathway for the generation of Th2 effectors in response to a subset of both allergens and helminths as well as innate eosinophilic inflammation. To this end, we have extended our previous observations by demonstrating that velvet grass pollen preparation contains a protein of approximately 30 kD with ribonuclease activity and is able to act on DC to promote Th2 differentiation.

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A TRAIL-REGULATED E3 UBIQUITIN LIGASE LINKS ALLERGEN AND RHINOVIRUS EXPOSURE TO ASThma VIA TARGETING A PROTEIN PHOSPHATASE

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Background: The majority of asthma exacerbations and wheezing illnesses are caused by rhinovirus (RV) infections. Thus RV infections are a major health burden because they contribute to asthma morbidity and mortality. The mechanisms that contribute to RV-induced exacerbation of allergic airways disease (AAD) in vivo are only poorly defined on a molecular and cellular level.

Methods: We employed a mouse model of house dust mite induced AAD and infected non-allergic and allergic mice with RV1B (minor group RV) to exacerbate disease. We used small interfering RNA and small molecules to specifically inhibit a E3 ubiquitin ligase and raise Protein Phosphatase activity, respectively.

Results: RV infection promoted neutrophilic airways inflammation in non-allergic mice. However in the presence of AAD, RV infection exacerbated eosinophilic inflammation and eotaxin-1 release. This was associated with enhanced airways hyperreactivity (AHR) and increased TRAIL expression in the airways. To further elucidate the molecular signals activated by RV to promote inflammation and AHR, we employed microarray technique and identified a TRAIL-regulated E3 ubiquitin ligase that targets a Protein Phosphatase for degradation. Inhibiting this TRAIL-regulated E3 ubiquitin ligase or raising the activity of the Protein Phosphatase in the airways abolished RV-induced AHR and eosinophilic airways inflammation, eotaxin-1 and CCL20 production, as well as levels of phosphorylated p38 MAPK. The TRAIL-regulated E3 ubiquitin ligase was also upregulated in airway epithelial cells from asthmatics and closely correlated with TRAIL expression upon ex vivo RV infection.

Conclusions: RV exacerbates AHR and eosinophilic airways inflammation in allergic mice, which can be prevented by inhibiting a newly identified TRAIL-regulated E3 ubiquitin ligase.

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EOSINOPHILS REGULATE LOCAL IMMUNITY DURING PARASITIC NEMATODE INFECTION

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Establishment of chronic infection is an important feature of many parasitic diseases, yet the immune regulatory mechanisms that support the long-term survival of parasites in infected hosts remain poorly understood. *Trichinella spiralis* initiates the chronic phase of infection when newborn, first-stage larvae infect skeletal muscle cells. We have discovered that eosinophils contribute to the ensuing myositis while simultaneously protecting larvae against immune-mediated destruction. Specifically, we find that growing larvae are killed in large numbers in Phil and dblGATA mice and, furthermore, that parasite survival improves when eosinophils are restored to such mice. Thus, the long-standing paradigm of eosinophil toxicity in nematode infection requires reevaluation, as eosinophils appear to regulate the immune response in a manner that supports chronic infection and insures worm survival in the host population. Our goal is to elucidate the properties and actions of eosinophils that enable them to sustain infection. We have found that eosinophils promote local recruitment of lymphocytes and activation of macrophages that protect parasites during a vulnerable phase of growth.

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DIAGNOSIS AND TREATMENT OF LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROME: STATE OF THE ART AND PERSPECTIVES IN 2011

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Lymphocytic variant hypereosinophilic syndrome, a disorder characterized by persistent clonal expansion of non-malignant T cells producing eosinophil growth factors, was first described as a case report more than 15 years ago. This patient, who fulfilled the diagnostic criteria for hypereosinophilic syndrome and in whom a population of IL-5 producing CD3-CD4+ T cells was detected in peripheral blood by flow cytometry, eventually developed T cell lymphoma and died. Since then, a number of reports have confirmed that hypereosinophilia is driven by in vivo activated T cells in a significant proportion of patients with HES, and in vitro studies have resulted both in an increased understanding of the biology of these cells, and in the identification of TARC as a useful diagnostic biomarker. However, besides IL-5, no clonal T cell-specific targets which could serve as a basis for development of innovative treatment strategies for lymphocytic variant hypereosinophilic syndrome have emerged from these studies. The recent characterization of the transcriptional profile of these CD3-CD4+ T cells before, and at the time of malignant transformation, have provided some exciting new perspectives in terms of improving diagnostic tools for this disease variant, and elucidating the functional requirements of these cells as well as the mechanisms involved in their transformation.

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NEW CLASSIFICATION OF EOSINOPHIL HEMATOPOIETIC DISORDERS:
REPORT FROM VIENNA MEETING

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Professor Peter Valent organized a Working Conference on Eosinophil Disorders and Syndromes that was held in Vienna, Austria May 27-28, 2011.

Invited participants included American and European individuals with expertise in the myeloid and other malignancies associated with eosinophilia and those with expertise in the diverse eosinophilic disorders that have been categorized as hypereosinophilic syndromes (HESs), the latter based in good part on prior workshops and publications arising from International Eosinophil Society consensus statements.

Goals of the Working Conference were in part focused on providing suggested modifications to the current WHO classification of myeloid disorders, including those with eosinophilia. The Working Conference was highly successful; and there was a consensus amongst hematologists/hematopathologists and IES immunologist/allergist members on how to integrate myeloproliferative HES and lymphoproliferative HES diseases into a new outline of myeloid neoplasms. Details of the revised classification scheme and associated diagnostic criteria and tests will be considered and further refined by the Working Conference.

On behalf of Professor Valent and the other participants in the Working Conference on Eosinophil Disorders and Syndromes, considerations of eosinophilic disorders arising from the Working Conference will be presented.
GASTROINTESTINAL EOSINOPHILS—FRIEND OR FOE?

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Most studies focus on eosinophil’s roles in the initiation and progression of inflammatory responses and the modulation of innate and adaptive immunity. This is especially true in the gastrointestinal (GI) tract where investigations examine eosinophil’s role in allergic and inflammatory diseases. But the fact that eosinophils are resident cells of the GI tract lends support for their potential role maintaining GI health. Studies in other organ systems provide the rationale for speculating on eosinophils impact and relationships with other resident epithelial cells, immune cells, neurons and fibroblasts that may bear functional influences on maintaining the health of intestinal mucosa. The talk will address potential roles for eosinophils in the GI tract addressing potential physiologic functions.

References:
MONOCYTE/MACROPHAGE-CCL11-EOSINOPHIL AXIS IN INFLAMMATORY BOWEL DISEASES

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Background: Elevated levels of eosinophils have been observed in colonic biopsy samples from pediatric and adult patients with ulcerative colitis (UC)1-3. Increased numbers of this cell and eosinophil-derived granular proteins MBP, ECP, EPO, and EDN have been shown to correlate with morphological changes to the gastrointestinal (GI) tract, disease severity, and GI dysfunction4. Consistent with these clinical observations, experimental studies have provided corroborative evidence of a pathogenic role for eosinophils in both chemical and spontaneous murine models of inflammatory bowel diseases (IBD). We have previously performed gene profiling on a cohort of pediatric UC patients at the time of diagnosis and identified a significant up regulation of CCL115. Importantly, CCL11 levels correlated with tissue eosinophil numbers, which in turn correlated with the UC Histologic Index of Severity (UCHIS). Experimental studies employing Ccl11-/- mice revealed a critical role for CCL11 in eosinophil recruitment into the colon. While a link between CCL11 and eosinophils in IBD has been established, the cellular source of CCL11 and molecular regulation of CCL11 expression in UC has not yet been delineated.

Objectives: Define the cellular source and molecular regulation of CCL11-dependent eosinophilic inflammation in experimental colitis.

Methods: Male and female, 6- to 8-week-old strain-, age- and weight-matched ccr2-/- (C57BL/6), ccl2-/- (C57BL/6), C57BL/6, BALB/c, and ccl11-/- (BALB/c) 6, cx3cr1GFP/+ 7 were administered dextran sodium sulphate (DSS) (40-45 kDa) in the drinking water as a 2.5%-5% (wt/vol) solution for up to 8 days. Disease monitoring and histopathologic changes in the colon were scored as previously described5. Cellular and biochemical analyses of leukocyte recruitment and cytokine production in the colon were determined by flow cytometry and ELISA. Bone marrow (BM) chimera techniques were used to distinguish the contribution of hematopoietic and non-hematopoietic cell-derived CCL11 in DSS-induced colonic eosinophilic inflammation.

Results: Employing a model of DSS-induced colitis and bone marrow (BM) chimera techniques, we show that hematopoietic cell-derived CCL11 is required for DSS-induced colonic eosinophilic inflammation. DSS exposure promoted the recruitment of F4/80+CD11b+CCR2+Ly6Chigh inflammatory monocytes to the colon, and the level of F4/80+CD11b+CCR2+Ly6Chigh colonic monocytes/macrophages positively correlated with colonic eosinophilic inflammation. Phenotypic analysis of purified Ly6Chigh intestinal inflammatory macrophages revealed that these cells express both M1- and M2-associated genes, including Il6, Ccl4, and Cxcl2 and Arg1, Chi3l3, Ccl11, and Il10, respectively. Ablation of DSS-induced F4/80+CD11b+CCR2+Ly6Chigh monocyte recruitment was associated with decreased intestinal CCL11 expression, colonic eosinophilic inflammation, and DSS-induced histopathology. Consistent with these observations, immunofluorescence analysis identified that CCL11 expression was restricted to CD68+ intestinal macrophages and the basolateral compartment of intestinal epithelial cells in pediatric UC.

Conclusions: These studies demonstrate that inflammatory monocyte/macrophage-derived CCL11 drives colonic eosinophilic inflammation in experimental IBD and provide strong rationale for the use of therapeutic agents targeting eosinophils and eosinophil regulatory molecules for the treatment of IBD, particularly UC.

References:

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MOUSE MODELS OF EOSINOPHILIC EOSPHAGITIS

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Summary / Abstract
Eosinophilic esophagitis is a complex disease characterized by eosinophilic infiltration in the esophagus. The etiology, involving genetic and environmental factors, and the pathogenesis of eosinophilic esophagitis are difficult to delineate in human patients due to the invasiveness of the procedures and the scarcity of the samples. Animal models have thus been developed to get insight into the disease pathogenesis. Using different strategies (allergen sensitization, cytokine instillation, transgenic animals), several models, derived or inspired from allergic disease models, have been established to attempt to reproduce the human eosinophilic esophagitis disease parameters. In this talk, the strengths and limits of these different models will be described as well as the knowledge gained in genetically modified animals.
INDUCTION AND ACTIVATION OF INVARIANT NATURAL KILLER T CELLS IS CRITICAL IN THE PATHOGENESIS OF EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) is a recently described chronic allergic inflammatory disorder whose basic pathogenesis is not well understood.

Objective: To test the hypothesis that iNKT cell responses are critical for the initiation and progression of EoE.

Methods: Quantitative PCR and ELISA analyses were performed to examine protein and transcript levels in experimental or human EoE tissue samples. Tissues from CD1d deficient and wild type mice were also examined. Tissue iNKT cell and CXCL16 protein and eosinophilia was determined by immunofluorescence and immunostaining using specific respective antibodies and flow cytometry for cell-surface receptors.

Results: We show that iNKT cells and their specific chemokine CXCL16 are induced in human and experimental EoE. CXCL16 is primarily derived from esophageal epithelial cells and correlates with the levels of esophageal eosinophils. Notably, iNKT cell- deficient CD1d-null mice are protected from the induction of experimental EoE; whereas, NK cell depleted mice exhibit full disease following allergen challenge. Interestingly, in vivo activation of human iNKT cells by the α-galactosylceramide analog PBS57 is sufficient to induce esophageal eosinophilia. Mechanistically, we show that PBS57 exposed human iNKT cells produce eosinophil active IL-5 and IL-13 via STAT5 activation.

Conclusion: Taken together, these findings provide evidence that iNKT cells have an critical pathogenic role in the initiation and progression of human and experimental EoE providing new opportunities for therapeutic strategies.
INVITED SPEAKER ABSTRACTS

GENOMIC AND MICRONRNA ANALYSIS OF EOSINOPHILIC ESOPHAGITIS

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Eosinophilic Esophagitis is a chronic allergic inflammatory disorder of the esophagus that is compounded by both genetic predisposition and aberrant responses to environmental antigens, particularly those derived from food. Recent advances in understanding the intrinsic (genetic) and extrinsic (environmental) components and the key molecular pathways and antigenic triggers illustrate the complex nature of this emerging disease. Expression profiling of mRNA and microRNA transcripts, as well as whole genome analysis of high-density genetic variants, have identified key etiological steps that provide novel insight into disease mechanisms and entry points for therapeutic strategies. Emerging data demonstrate the interplay of epithelial cells, mast cells and eosinophils, Th2-associated cytokines including IL-13 and TSLP, as well as acquired and inherited impairment of barrier function.
REMODELING, EOSINOPHILS, AND EOSINOPHILIC ESOPHAGITIS

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Background:
Eosinophilic esophagitis (EoE) is an increasingly recognized disorder that is characterized by significant, diffuse esophageal eosinophilia in response to dietary and environmental antigens [1]. EoE is associated with clinical dysphagia, dysmotility, and esophageal tissue remodeling. Remodeling features include angiogenesis and fibrosis with increased numbers of cells expressing the pro-fibrotic factor TGFβ1 [2]. IL-5 can induce features of both epithelial and subepithelial remodeling in animal models.3

Objective:
To assess the potential effects of eosinophils and IL-5 on remodeling features in pediatric EoE.

Methods:
Pediatric EoE patient biopsies were assessed for eosinophils, mast cells, and features of tissue remodeling using quantitative immunohistochemistry. In vitro assays using primary esophageal smooth muscle cells were completed to assess the impact of inflammatory cell derived TGFβ1.

Results:
Patients with EoE have elevated numbers of eosinophils in the epithelium, lamina propria, and smooth muscle bundles of the esophagus. Both eosinophils and mast cells produce TGFβ1. TGFβ1 causes contraction of esophageal smooth muscle cells in vitro using collagen gel contraction assays [4]. Pediatric patients treated with anti-IL-5 (mepolizumab) who demonstrate decreases in epithelial eosinophilia following therapy also have diminished numbers of epithelial mast cells.

Conclusions:
Eosinophilia occurs in both the esophageal mucosa and submucosa in pediatric EoE patients. Smooth muscle eosinophilia and mastocytosis may affect smooth muscle contraction in a TGFβ1 dependent manner. Anti-IL-5 therapy can significantly reduce esophageal inflammation as well as epithelial mastocytosis, suggesting that eosinophils and/or IL-5 may promote mast cell survival in EoE.

References:

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MEDICAL TREATMENT OF EOSINOPHILIC ESOPHAGITIS: NEW INSIGHTS

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Background Eosinophilic Esophagitis (EoE) is a clinico-pathological defined condition characterized by symptoms attributed to esophageal dysfunction in combination with a chronic, eosinophil-predominant esophageal infiltration. EoE is the leading form of Eosinophilic Gastrointestinal Diseases (EGID). Topical corticosteroids have shown to be an efficient short-term therapy in children and, on an anecdotal base, in adults. However, EoE is a chronic disease and long-term therapeutic management is therefore required. Moreover, corticosteroid-resistant patients exist and alternative treatment strategies are lacking.

Objectives The purposes of the three studies were 1.) To assess the efficacy of topical corticosteroids to induce a remission in adults with active EoE (induction = BEE-I trial); 2.) To assess the efficacy of topical corticosteroids in maintaining quiescent EoE in remission (maintenance = BEE-M trial); and 3.) To test a therapeutic alternative using IL-5 blockade with mepolizumab to specifically target eosinophils (mepolizumab = MEE trial).

Methods BEE-I, BEE-M and MEE were randomized, double-blind, placebo controlled trials, including 36, 28 and 11 adult EoE-patients, respectively. In BEE-I, the topical corticosteroid budesonide was swallowed in a dose of 1 mg bid for 2 weeks and in the subsequent BEE-M trial, budesonide was swallowed in a dose of 0.25 mg bid for another 50 weeks. In MEE, patients received two infusions with 750 mg mepolizumab one week apart; those not in complete remissions after 8 weeks received two further infusions with 1500 mg mepolizumab 4 weeks apart. The effect of the study-medications was assessed in all trials clinically, endoscopically, histologically, and via blood and tissue biomarkers.

Results A 2-wk induction-treatment with budesonide brought 72.2% of patients with active EoE either in complete (<5 eos/hpf) or in partial (5-20 eos/hpf) remission. The subsequent 50-wk maintenance-treatment was able to keep 50% of patients in complete or at least in partial remission. Budesonide was significantly more efficient than placebo as both induction and maintenance therapy. Mepolizumab induced a marked reduction of mean esophageal eosinophil numbers (-54%) compared with the placebo (-5%) within four weeks after initiation of treatment. Blood eosinophil numbers also significantly declined in the mepolizumab but not in the placebo group (p=0.006). Mepolizumab reduced tenascin C (p=0.039) and TGF-beta (p=0.05) expression in the esophageal epithelial layer 13 weeks after initiation of treatment. Clinically, no impressive improvement of symptoms was seen, although a trend to an improvement was observed between 4 and 13 weeks after initiation of mepolizumab therapy. Budesonide and mepolizumab were well tolerated and no relevant adverse events occurred.

Conclusions A 2-wk course with the topical corticosteroid budesonide is highly effective in inducing clinically and histologically remission in adolescents and adults with active EoE. Subsequent low-dose budesonide is more effective than placebo in maintaining EoE in histologic and clinical remission. Signs of esophageal remodeling showed a trend toward normalization. Mepolizumab significantly reduced eosinophil numbers in both blood and esophageal tissues in patients with active EoE. Changes in the expression of molecules associated with esophageal remodeling were reversed by the anti-IL5 treatment. However, minimal clinical improvement was achieved only. Budesonide and mepolizumab had a favorable safety profile. The optimal long-term management of EoE remains to be determined.

References

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EOSINOPHILS AND DERMATOLOGIC DISEASES

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Background: The functional role of eosinophils has been attributed to host defense, immunomodulation and fibrosis. Under physiological conditions, eosinophils are absent in the skin, but they are found in a broad spectrum of diseases, including allergic, infectious, autoimmune and neoplastic diseases. Recently, eosinophil extracellular traps (EETs), structures containing DNA in association with eosinophil granule proteins able to bind and kill bacteria, have been reported.

Results: By immunofluorescence staining and laser scanning microscopy, eosinophils releasing DNA together with ECP were detected in infectious skin diseases, such as ectoparasitosis and larva migrans. Further, EETs were observed in allergic/reactive diseases (Wells’ syndrome, hypereosinophilic syndrome, positive reaction of atopy patch test, allergic contact dermatitis, drug hypersensitivity) and autoimmune diseases (bullous pemphigoid, pemphigus foliaceus, dermatitis herpetiformis). By analyzing the cytokine expression in tissue specimens from various skin diseases, eosinophils were shown to produce proinflammatory (TNF-α), Th1-type (IFN-γ), Th2-type (IL-5, IL-13, IL-25), regulatory (IL-10), and profibrotic cytokines (IL-6, IL-11), as well as eotaxins (CCL-11, CCL-24, CCL-26) and MMP-9. However, the patterns of cytokine production varied in different diseases suggesting distinct functions of eosinophils depending on the underlying pathomechanism of the disease.

Conclusion: Thus, by releasing EETs, cytokines and mediators, eosinophils might actively be involved in pathogen removal, regulation of inflammation and/or remodeling in skin diseases. A better understanding of their role in skin pathology will help to identify new disease subgroups and possibly new therapeutic targets.
Eosinophils support the long term survival of plasma cells in the murine bone marrow

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Background: In the bone marrow the underlying network of reticular stromal cells provides a micro-environment which supports long-term survival of plasma cells. The mutual interaction between stromal cells and plasma cells is a prerequisite for the longevity of plasma cells. In addition, plasma cells require survival factors such as the cytokines APRIL and IL-6.

Objectives: We asked the question which cell types in the bone marrow provide the plasma cell survival factors.

Methods: To analyze the development and the maintenance of long lived plasma cells, animals were immunized with the Alum precipitated T cell dependent antigen 2-phenyl-oxazolone. Bone marrow cell suspensions were analyzed by flow cytometry and APRIL and IL-6 high producing cells determined. In addition, bone marrow tissue sections were prepared and the plasma cell survival niche analyzed by staining with antibodies specific for plasma cells eosinophils and stromal cells. Eosinophils were sorted from the bone marrow of naive mice or 6 days after 20 immunization with soluble antigen. To determine whether eosinophils support plasma cell survival, cells were co-cultured for 48h and the percentage of surviving plasma cells determined. Eosinophils were depleted by 3 injections of Siglec-F specific antibodies within one week. 6 days after the last injection the number and percentage of Annexin-V+ plasma cells was determined. The number of plasma cells in the bone marrow was measured using ELISPOT and flow cytometry.

Results: In the murine bone marrow we find eosinophils in direct contact with plasma cells suggesting that eosinophils are an essential part of the plasma cell survival niche. Co-culturing of eosinophils and plasma cells demonstrate that eosinophils support plasma cell survival by secretion of APRIL and IL-6. Eosinophils are the key providers of the plasma cell survival factors and as a consequence, plasma cells rapidly go into apoptosis when eosinophils are depleted. In addition, eosinophils are required for the accumulation and the development of mature long lived plasma cells in the bone marrow.

Conclusions: It is more than surprising that the long term humoral immunity is dependent on a short lived cell of the innate immune system. This predicts that the plasma cell survival niche is a dynamic niche where dying eosinophils are constantly replaced by new ones. Thus, a continuous interaction between eosinophils, plasma cells and the underlying reticular stromal cells is required to ensure the longevity of plasma cells in the bone marrow. In the long term these active processes may guarantee a higher stability and make the niche less vulnerable.


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IDENTIFICATION OF INNATE IL-5-PRODUCING CELLS THAT PROMOTE EOSINOPHIL RECRUITMENT AND CONTRIBUTE TO ANTITUMOR IMMUNITY

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Background: IL-5, a member of Th2 cytokines, is essential for eosinophil regulation. In animal models of allergic diseases or helminth infection, IL-5 induces a massive proliferation of eosinophil progenitors in the bone marrow, promotes eosinophil recruitment and prolongs eosinophil survival in local tissues.

Objectives: We aimed at identifying and characterizing IL-5-producing cells to investigate their regulatory mechanisms and functions in relation to eosinophil regulation.

Methods: We generated an IL-5 reporter mouse in which IL-5-producing cells are detected by expression of a fluorescent protein. Flow cytometry was used to analyze cells in immune-related tissues and organs in those mice. Since IL-25 and IL-33 were well-known to mediate type 2 innate immunity including eosinophil recruitment, these cytokines were intraperitoneally injected into IL-5 reporter mice to assess their effect on IL-5 production. One of the functions of eosinophils has been shown to be prevention of tumor occurrence, in which IL-5 is suggested to be largely involved. We therefore performed an experimental model of tumor metastasis in IL-5 deficient and IL-5 reporter mice. A melanoma cell line (B16F10) was employed for tumor metastasis analysis.

Results: Flow cytometry analysis revealed that relatively abundant IL-5-producing non-T lymphoid cells resided in the intestine, peritoneal cavity and lungs in naive mice. The numbers of so-called innate IL-5-producing cells were calculated to be largest in the lung. Characterization of lung innate IL-5-producing cells revealed that they were lineage negative and expressed c-kit, Sca-1 and T1/ST2, a subunit of IL-33R. We examined the effect of IL-25 and IL-33 and demonstrated that both cytokines potently induced innate IL-5-producing cells and recruited eosinophils to the lung in an IL-5-dependent manner. In analysis of lung tumor metastasis, IL-5 deficient mice showed severely elevated tumor metastasis and innate IL-5–producing cells were increased after tumor injection. IL-5 deficient mice also exhibited a shortage of lung eosinophils in the steady state and impaired eosinophil recruitment in response to tumor injection.

Conclusions: We have identified innate IL-5-producing cells that contribute to accumulating and recruiting eosinophils to the lung in response to IL-25 and IL-33 as well as tumor invasion. They may also be capable of protecting against pathogenic infection at pulmonary sites. These newly identified innate IL-5-producing cells thus play a role in immune surveillance and might contribute to the development of novel immunotherapies for infection and cancer.

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ASTHMA AND EOSINOPHILS: CAN SOME CONTROVERSIES BE OVERCOME?

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The definition of asthma is commonly misunderstood. Some say it cannot be defined while others regard it as a syndrome or as a disease entity. These are inappropriate. While its characteristics can include symptoms, airway inflammation and abnormalities of airway function, it is best defined and diagnosed by its physiological abnormality of airflow limitation that varies over short periods of time. This is recognized by beta-agonist reversibility, airway hyper-responsiveness or an increase in the diurnal variation of peak expiratory flow. This definition is in line with those of other airway diseases with no unifying cause, eg. chronic obstructive pulmonary disease (defined by its abnormality of function) and with bronchiectasis or emphysema (defined by pathology), with which asthma can coexist. Asthma has many phenotypes, the recognition of which is useful to personalize treatment. It also has several endotypes, the study of which might lead to a better understanding of the pathophysiology.

The airway inflammation component of asthma is commonly eosinophilic but it can be non-eosinophilic or neutrophilic or a combination of these and it varies from one time to another due to different environmental causes. Its recognition by sputum quantitative inflammatory cell counts provides the most specific, comprehensive and discriminative test. While this has been touted to be complex, expensive and impractical, an understanding of what is required in the performance of the procedure illustrates the opposite. Sputum cell counts should not be regarded as routine in the treatment of asthma but as especially required when disease is severe, difficult or complicated.

Sputum eosinophils when increased predict benefit from corticosteroid treatment and when absent in patients treated with corticosteroid suggests that the dose is excessive. Hence, serial measurements can be used to identify the minimum dose required to maintain control and to reduce clinical exacerbations.
EOSINOPHILS AND ASTHMA: A LINK TO EXACERBATIONS AND REMODELING

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Eosinophils are a prominent feature of asthma as they are found in increased numbers in the peripheral circulation, sputum, and airway mucosa. Moreover, the presence of eosinophils, especially in airway locations, is often associated with features of asthma severity. The treatment of asthma, especially with corticosteroids is associated with the control of symptoms, improvement in pulmonary functions, and reduced frequency of exacerbations along with a suppression of eosinophils. These associations and correlations have, for years, suggested that the eosinophil is not only a marker for the presence of asthma and its severity, but also a pivotal cell in the pathogenesis of this disease.

With the use of monoclonal antibodies against IL-5, however, a more defined, and circumscribed, picture of the eosinophil in asthma has emerged. Two aspects of asthma appeared to be closely linked to the presence of eosinophils: exacerbations and progressive loss of airway function, i.e., remodeling. In this presentation, the role and contribution of eosinophils as a marker for the susceptibility for exacerbations and remodeling will be discussed, as well as phenotypes of asthma where this cell may be linked to the pathophysiology of asthma.
REGULATION OF EOSINOPHIL PROGENITORS IN ALLERGIC ASTHMA

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The onset and maintenance of allergic asthma is associated with the activation of hemopoietic processes. This has been studied in detail in an experimental model of allergic asthma. Following allergen inhalation challenge, eosinophils appear in the circulation upon the efflux of mature and immature populations from the bone marrow. The accumulation of eosinophils within the airway tissue occurs as a response to locally generated signals which induce the production of eosinophils in the bone marrow, differentiate progenitor cells, direct the migration of eosinophils and their progenitors, and increase survival and effector functions of these cells. Progenitor cells are emerging in the literature as effector cells which can migrate to inflamed tissue where they can rapidly proliferate in response to allergic stimuli.

Allergic asthma is primarily a Th2-mediated disease, and cytokines driving eosinophilia are the Th2 cell products interleukin (IL)-3, IL-5 and GM-CSF, which signal through specific high-affinity α-chain receptors linked to a common β-chain. Although each of these cytokines function as eosinophil growth factors which promote the formation of eosinophil/basophil (Eo/B) colony-forming cells (CFU) in functional assays, IL-5 is the only cytokine necessary for mobilization of eosinophil progenitors from the bone marrow and their terminal differentiation. This discovery was instrumental for development of drugs targeting the eosinophil. The rapid increase in expression of IL-5Rα on CD34+ cells from bone marrow of atopic asthmatics post-allergen challenge clearly demonstrates that progenitor cells in these subjects are “primed” to respond to IL-5.

Anti-IL-5 treatment in mild atopic asthmatic subjects has been shown to induce a reduction of airway eosinophils, arrest bone marrow eosinophil maturation, and decrease eosinophil progenitors in the bronchial mucosa. Initial clinical trials of IL-5 blockade in patients with asthma were not successful in demonstrating clinical efficacy, however a number of issues may have contributed to the failure of these studies, including incomplete depletion of tissue eosinophils and their granule products. Although controversial, chemokines such as eotaxin may also play a role in the differentiation of progenitor cells since CD34+ progenitor cells are found to express CCR3 which is upregulated in a Th2 environment. It is hypothesized that redundant cytokine mechanisms can compensate for IL-5 deficiency, and combined therapies may be more effective for suppression of eosinophils in diseases such as allergic asthma.

One such therapy is currently being tested in clinical trials of allergic asthma. TPI-ASM8 is a combination of two antisense oligonucleotides, one blocking translation of the IL-3/5/GM-CSF common beta chain, and the other blocking translation of CCR3. Treatment with TPI-ASM8 should inhibit many of the signals critical for eosinophilopoiesis, and eosinophil migration activation and survival. Following allergen challenge in mild atopic asthmatic subjects, inhaled TPI-ASM8 inhibited accumulation of mature eosinophils and CD34+IL-5Rα+ cells in the sputum, in conjunction with inhibition of the late asthmatic response. Therapies such as TPI-ASM8 which target these eosinophil progenitor cells and hemopoietic processes may be effective in the control of allergen-induced eosinophilic airway inflammation.
HUMAN STUDIES ON EOSINOPHILS AND ALLERGIC TISSUE REACTIONS

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Human allergic tissue reactions can be studied by sampling in the airways or skin following provocation by either allergen inhalation challenge or direct intracutaneous administration to sensitised atopic individuals. A single injection of the relevant allergen is characteristically followed an immediate phase, a delayed phase and a repair (or remodelling) phase. Eosinophils appear early in the tissues and in the skin are significantly raised at 1 hour, peak between 6 and 8 hours after which their numbers are usually completely resolved by 72 hours. Eosinophils are recruited into the skin largely by mast cell-derived eotaxin but in the lung CD4+ Th2 cells also contribute to accumulation, presumably through IL-5/bone marrow mechanisms. Eosinophils do not appear to be essential for the development late-phase reactions in either the skin or airways since anti-IL-5 had no effect on the late-phase skin or asthmatic reactions. Furthermore in the lung isolated late-phase reactions, independent of an immediate response, can be elicited by peptide-derived allergens, a situation where eosinophilia is not prominent. Late-phase reactions in the lung and skin appear to be dependent on the elaboration on vasodilator and permeability factors. There is particular interest in calcitonin gene related peptide (CGRP) since CGRP protein and mRNA+ cells have been identified in the skin and the airways at the height of the late phase response. In the skin the prominent CGRP+ cell is the neutrophil and their numbers mirror the formation and resolution of the late-phase response. Thus late-phase reactions may be examples of neutrophil-dependent permeability previously described in experimental animals and which require a combination of vasodilator and vascular permeability factors. The repair process, often referred to as remodelling in asthma, is a complex process in which the eosinophil appears to play some role. Eosinophil depletion studies show a significant reduction in markers of airway remodelling such as reticular basement membrane procollagen III, lumican and tenasin. Type IV collagen remained unchanged. These observations were associated with reduction in numbers and percentage of airway eosinophils expressing TGF-β1.

Eosinophils also express a number of other remodelling associated agents, including VEGF, β-FGF, NGF, osteopontin and BMP-7. Remodelling does not appear to depend on persisting eosinophilic inflammation since airway eosinophils were significantly increased 24 hours post-challenge along with remodelling but returned to baseline levels at 7 days when remodelling was sustained and maximal. In conclusion, eosinophils, whilst prominent in late phase responses probably contribute little to the vascular oedema characteristic of this phenomenon. In contrast eosinopils appear to contribute to repair processes associated with allergic inflammation but precisely how is still unclear. Long-term eosinophil depletion studies are required before the exact contribution by eosinophils to allergic inflammation and airway remodelling can be established.
NEW INSIGHTS INTO THE ROLES OF EOSINOPHILS IN CHRONIC RHINOSINUSITIS

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Chronic rhinosinusitis (CRS) is a heterogeneous chronic disease typically characterized by intense recruitment and activation of eosinophils in sinonasal tissues. The association of eosinophilia with CRS has been known for a century. Different forms of CRS have variable levels of elevations of eosinophils, with the non-polypoid form (CRSsNP) having the least elevated eosinophil levels, the polypoid form having higher eosinophils (CRSwNP) and Samter’s triad, which is defined as the coexistence of CRSwNP, asthma and aspirin sensitivity, having the highest levels of eosinophils. Recruitment and survival of eosinophils is maintained within nasal polyps by well-known pathways, including expression of important inflammatory molecules such as endothelial VCAM-1, CCR3 active chemokines and survival promoting cytokines such as IL-5. Based on recent results demonstrating polyp shrinkage with anti-IL-5 antibodies, it can be concluded that eosinophils likely contribute to structural changes such as formation of nasal polyps. Recent studies by Bachert et al and by our group suggest that CRSwNP is accompanied by the presence of alternatively activated macrophages (AAM), which agrees with earlier studies suggesting a Th2 dominance in CRSwNP. We have recently found that the chemokine CCL23, which is an agonist of CCR1, is significantly elevated in nasal polyps from patients with polypoid CRS compared to tissues from patients with non polypoid CRS or healthy subjects. Immunohistochemical analysis revealed CCL23 expression in inflammatory cells and immunofluorescence demonstrated substantial co-localization of CCL23 with ECP within eosinophils. The concentration of CCL23 in nasal polyp tissue strongly correlated with the concentration of ECP, suggesting that eosinophils are a major CCL23 producing cell type in nasal polyps. The most profound elevations of CCL23 protein were found in nasal polyps from patients with Samter’s triad in association with the most intense eosinophilia. The levels of CCL23 in nasal polyp tissue strongly correlated with the levels of CCR1. Production of CCL23 by eosinophils may result in the recruitment of CCR1 positive inflammatory cells including monocytes and macrophages. Future studies will be required to determine whether this is a feedback mechanism mediating repair and regulating inflammation or whether eosinophil-derived CCL23 plays an important mechanistic role in the pathogenesis of eosinophilic CRSwNP.

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AIRWAY EXPOSURE TO FUNGAL ANTIGENS INDUCES INNATE EOSINOPHILIA MEDIATED BY A NOVEL TH2-TYPE LYMPHOID CELL SUBSET

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Abstract: Innate immunity provides the first line of response to invading pathogens and a variety of environmental insults. Epithelium-derived cytokines, such as TSLP and IL-33, likely play critical roles in regulating Th2-type immunity in the airways. Here we describe a novel subset of leukocytes that mediates innate Th2-type immunity and eosinophilia in the lungs. Airway exposure of naïve BALB/c mice to IL-33 results in rapid production of IL-5 and IL-13 and marked airway eosinophilia independently of adaptive immunity. We identified the IL-33-responsive cells that are lymphoid in morphology, lack lineage markers (i.e. Lin-), but highly express Thy1.2, CD25, CD44, IL-7Ra, Sca-1 and T1/ST2 (i.e. an IL-33 receptor subunit). Further, airways exposure of naïve mice to extracts of Alternaria alternata, a fungal allergen implicated in human asthma, induces airway release of IL-33 followed by innate production of Th2-type cytokines, airway eosinophilia, and pathological changes. The inflammatory responses are absent in Il7r-/- mice, but are reconstituted by transferring those innate lymphoid cells from wild-type mice. Thus, a novel subset of immune cells that responds vigorously to IL-33 and rapidly produces Th2-type cytokines is present in mouse lungs. These cells may provide an innate mechanism for Th2-type immunity in the airways and induction/exacerbation of asthma and allergic disorders.

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INVITED SPEAKER ABSTRACTS

A TALE OF TWO CELLS:
INTERACTIONS BETWEEN NERVES AND INFLAMMATORY CELLS IN THE LUNGS.

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Eosinophils were first reported around airway nerves in fatal asthma. Likewise, eosinophils are present around airway nerves in antigen challenged guinea pigs. In this model, eosinophil depletion or any treatment that shifts tissue eosinophils away from airway nerves inhibits airway hyperreactivity. Eosinophils are also present around nerves in the nose of humans with rhinitis, skin of humans with atopic dermatitis, optic nerve of mice with multiple sclerosis and motor nerves of dogs that are surgically damaged. In the lungs of people with fatal asthma, there are more eosinophils associated with nerves than with blood vessels or airway smooth muscle. Animal models demonstrate that the number of eosinophils associated with nerves is a better predictor of airway hyperreactivity than eosinophils in the bronchoalveolar lavage fluid.

Recruitment of eosinophils to nerves is a dynamic and active process. Following ozone, sequential waves of eosinophils move through the lungs, recruited by eotaxin and ICAM, both of which are made by airway nerves. The initial wave of eosinophils cluster around airway nerves, and directly cause airway hyperreactivity via secretion of the M2 muscarinic receptor antagonist, major basic protein, resulting in increased release of acetylcholine and increased bronchoconstriction. In vitro, eosinophils increase nerve branching, which may, in vivo, increase nerve density in tissues. In mice, airway neural reflexes are only present in the presence of eosinophils.

There are also beneficial effects to the eosinophil-nerve interaction. Several days after a single exposure to ozone, secondary waves of eosinophils enter the lungs. These eosinophils are beneficial and suppress airway hyperreactivity. While the mechanism of this effect is unknown in vivo, eosinophils have been shown to increase M2 receptor expression in neuronal cell lines and to decrease acetylcholine content, both of which could be mechanisms for suppressing neurotransmitter release. Eosinophils also express muscarinic receptors, and thus are capable of responding to neurotransmitters. There are preliminary data suggesting these muscarinic receptors contribute to eosinophil migration, but this has not been confirmed.

Thus, eosinophils and nerves are found in vivo in close proximity to each other. They share muscarinic receptors, and respond to cytokines, antagonists and chemokines secreted by each other. While the full extent of interactions between these two cells remains to be determined, the recurring presence of eosinophils around nerves in different organs, and direct link between eosinophil recruitment to nerves and airway hyperreactivity suggest these interactions will be important in both physiological and in pathological mechanisms.
VIRUSES, NERVES, AND EOSINOPHILS IN ASTHMA

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The role of eosinophils in virus induced asthma attacks may be a two edged sword. Where eosinophils are present in the airways, they may be activated by viral infections in a process that likely involves detection of viral RNA by TLR7. This may be potentiated by interferon-gamma, which increases eosinophil TLR7 expression. Once activated, the eosinophil exerts a potent antiviral effect both in vivo and in vitro. Multiple mechanisms may participate in the antiviral effects, including but not limited to ribonuclease activity of eosinophil derived neurotoxin and eosinophil cationic protein as well as generation of hypobromous acid by eosinophil peroxidase. The overall effect of virus-eosinophil interactions on virus induced airway hyperreactivity varies depending on the specific model being examined. Eosinophils can also be infected with airway viruses. Although this results in generation of viral RNA, whether infectious progeny virus is produce by infected eosinophils appears to vary with the specific virus. Thus there are multiple effects of the eosinophil in the virus infected airway, and the overall result in terms of airway function may be determined by the balance of the beneficial effects of limiting viral replication versus the detrimental effects of eosinophil activation and degranulation.

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NONINVASIVE ASSESSMENT OF ATOPIC DISEASES: METABOLOMIC PROFILING OF URINE USING NUCLEAR MAGNETIC RESONANCE (NMR) ANALYSIS

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Background: Management of asthma inflammation requires anti-inflammatory therapies. Existing methods for determining inflammation levels (i.e. lung function tests, sputum analysis, exhaled nitric oxide levels, and bronchoscopy) are not practical for most patients in a typical primary care setting. Metabolomics is the study of small molecules generated from cellular metabolic activity. Different inflammatory cells, including the eosinophil, possess different enzymes, thus metabolites reflect the activity of those unique cell types. We hypothesized that the unique cellular activity present in the asthmatic lung would be reflected in unique metabolites generated and excreted in the urine. The analytical method of 1D 1H-nuclear magnetic resonance spectroscopy (NMR) has the capacity to identify metabolites and chemicals in solution based upon their unique nuclear spin properties.

Objectives: To determine whether metabolites in the urine can be used to discriminate asthma, from non-asthma subjects or those with asthma exacerbation.

Methods: Clinical information and urine samples were collected from stable asthmatic patients at the Stollery Children’s Hospital pediatric outpatient asthma clinic and acute asthma from the Emergency Department (n=60 and 20, respectively, ages 4-16). Age and sex matched controls were also sampled to match each patient cohort. We correlated clinical presentation, age, sex, history/physical exam, medication dosage, atopic status, and lung function from each visit with urine NMR data. NMR urine spectra were collected on a 600MHz spectrometer and concentrations of metabolites determined using Chenomx NMR Suite software.

Results: Seventy metabolites were referenced to creatinine and analyzed by partial least squares discriminate analysis (PLS-DA). A combination of metabolites detected in the patients’ urine allowed for correct blinded identification of asthmatic children from healthy controls with a sensitivity of 94%. There was only a 5% false positive rate. Blinded NMR analysis also discriminated urine samples from stable asthmatic children in the clinic compared with those of asthma exacerbation in the emergency department again with 94% accuracy.

Conclusions: Nuclear magnetic resonance spectroscopy can identify metabolites that appear to be unique to children with asthma and appear to change with asthma severity. We believe this approach could lead to an improved noninvasive diagnostic for patients of all ages with asthma and potentially other respiratory disorders.

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Antagonists of IL-5 in Asthma

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Asthma is characterised by rapid variations in airflow obstruction, increased sensitivity to non specific irritants (airway hyperresponsiveness; AHR), chronic inflammation affecting the bronchial mucosa which is generally although not invariably eosinophilic, increased cough reflex and in long standing disease the development of airway damage (including bronchiectasis and fixed airflow obstruction). The way in which these various pathophysiological abnormalities relate to aetiology, symptoms and treatment response is complex and leads to considerable heterogeneity in disease expression. A persuasive concept that has greatly influenced the development of anti-inflammatory drugs in asthma has viewed the abnormalities in airway smooth muscle function that lead to variable airflow obstruction and increased AHR as being secondary to the eosinophilic inflammation. However it is increasingly clear that there is no necessary link between eosinophilic inflammation and airway dysfunction and that each can occur independently. The standard outcome measures of clinical trials of new drugs for asthma (AHR, FEV1 and day-to-day symptoms), relate closely to the airway dysfunction element of asthma, but not to the inflammatory component which is often clinically silent. In contrast eosinophilic airway inflammation is much more closely related to oral steroid responsive severe exacerbations of asthma which is pathologically caused by obstruction of the airway lumen with mucus and cellular debris. Drugs which block eosinophilic inflammation are likely to be seen to best effect in patients who suffer from eosinophil associated exacerbations. However the majority of studies of Th2 cytokine antagonists have not selected patients with this profile of disease or used severe exacerbations as a primary outcome measure. Thus the early studies of antibodies against IL-5 in particular mepolizumab developed by GSK, were disappointing in that they appeared to have no effect on standard measures of asthma despite markedly reducing the blood and sputum eosinophil count. However two clinical trials of mepolizumab that targeted patients with eosinophilic disease and used severe exacerbations as the primary outcome measure did show a benefit. Haldar et al found a greater than 40% reduction in severe exacerbations in patients recruited from a difficult asthma clinic and Nair et al in patients with oral steroid dependent disease using a steroid reduction design were able to show a marked steroid sparing effect and a reduction in severe exacerbations 1,2. A striking feature of the Haldar study was that severe exacerbations were prevented without any significant effect on AHR, FEV1 or symptoms emphasising the potential dissociation between airway dysfunction and inflammation in asthma. Patients with uncontrolled eosinophilic disease who suffer severe exacerbations are not rare as suggested by some commentators making up a significant part of a severe asthma population where they are a considerable clinical challenge.

These studies which provide direct evidence for an effector role for eosinophils in asthma have spurred the development of IL-5 antagonists and phase 2/3 studies of mepolizumab, reslizumab (an anti-IL-5 antibody originally developed by Schering-Plough) and MEDI-563 are underway. The latter drug is an antibody which binds to the IL-5 receptor and causes ADCC. IL-5 antagonists only reduce tissue eosinophils by about 50% at best. Although as indicated above current evidence suggest that eosinophils are not involved in causing airway dysfunction until the bronchial wall eosinophilia is ablated, which may occur with MEDI-563, it is not possible to make definitive statements about the exact role of eosinophils in asthma. In summary IL-5 antagonists have clearly demonstrated that eosinophils mediate at least some aspects of asthma and are likely to be highly effective drugs for a subset of severe asthmatics with eosinophil driven exacerbations.

INVITED SPEAKER ABSTRACTS

BENRALIZUMAB, A HUMANIZED ANTI-INTERLEUKIN 5 RECEPTOR-ALPHA MONOCLONAL ANTIBODY, WITH ENHANCED ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY FUNCTION, IN DEVELOPMENT FOR THE TREATMENT OF ASTHMA

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Peripheral blood eosinophilia and lung mucosal eosinophil infiltration are hallmarks of bronchial asthma. Interleukin-5 is a critical cytokine for eosinophil maturation, survival and mobilization. Previous attempts to target eosinophils for the treatment of asthma by means of IL-5 neutralization have resulted in the reduction of acute exacerbations and corticosteroid sparing in asthmatics with elevated sputum eosinophilia, although the removal of airway eosinophils was only partial. We have developed a novel humanized anti-interleukin-5 receptor α monoclonal antibody with enhanced effector function (benralizumab) that potently depletes circulating eosinophils. Benralizumab binds to an epitope on the interleukin-5 receptor α that is in close proximity to the interleukin-5 binding site, and it inhibits IL-5-mediated cell proliferation. Benralizumab potently induces antibody-dependent cell-mediated cytotoxicity of both eosinophils (EC50=0.9 pM) and basophils (EC50=0.5 pM) in vitro. Single intravenous dose administrations of benralizumab to mild atopic asthmatics (0.0003-3 mg/kg) resulted in a transient decrease of mean peripheral blood eosinophil levels below detection levels within 24h, that lasted for several weeks. In this non-placebo controlled study, single escalating doses of benralizumab were associated with reductions in transient white blood cell counts, nasopharyngitis, and transient increased blood creatine phosphokinase levels.

A follow up study evaluated the safety and tolerability profiles of escalating multiple subcutaneous (SC) doses of benralizumab (25, 100 and 200 mg) in adult subjects with asthma. No serious adverse events occurred during the study and no subject experienced injection-site reaction or was discontinued due to an adverse event. Further, SC administration of this antibody depleted peripheral blood eosinophils in all active cohorts to levels below detection by day 7; eosinophils remained undetected through day 161. The safety, pharmacokinetic, and pharmacodynamic profiles of benralizumab demonstrated in these studies support continued development of this antibody. Thus, benralizumab may provide a novel approach for the treatment of asthma via active antibody-dependent cell-mediated depletion of eosinophils rather than through passive removal of interleukin-5.
TARGETING EOSINOPHIL-SPECIFIC SIGLECS

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Background: Human and mouse eosinophils selectively express the ITIM-containing, pro-apoptotic surface molecules Siglec-8 and Siglec-F, respectively. These two siglecs are functional paralogs capable of binding the same glycan ligand, namely 6’-sulfated sialyl Lewis X and its related non-fucosylated form.

Objectives: To explore mechanisms by which Siglec-8 and Siglec-F engagement results in apoptosis and to characterize the natural tissue ligands capable of binding these siglecs.

Methods: Human eosinophils were isolated from peripheral blood using immunomagnetic negative selection. Mouse eosinophils were obtained from blood or spleens from IL-5 transgenic or other mice or grown from bone marrow precursors of wild-type mice or mice lacking intracellular molecules putatively involved in Siglec-F signaling such as SHP-1 and NADPH oxidase. In some cases, pharmacologic inhibitors were used to explore siglec engagement-induced production of ROS using standard assays. Finally, immunohistochemistry and western blotting were used to detect and characterize Siglec-F and Siglec-8 ligands in lung tissues and epithelial cell cultures.

Results: Human eosinophils primed with IL-5 produced significant quantities of ROS following Siglec-8 engagement that was dependent on PI3 kinase but not ERK kinase 1. In contrast, engagement of Siglec-F, even after cytokine priming, did not result in detectable ROS production. Furthermore, Siglec-F engagement resulted in similar levels of caspase-dependent apoptosis even in eosinophils from mev mice deficient in SHP-1 and Ncf mice deficient in the p47 subunit of NADPH oxidase. In separate studies, lung tissue sections were found to bind Siglec-F-Ig fusion proteins. Binding was sialidase sensitive and was absent in mice deficient in the St3gal3 sialyltransferase. The plant lectin MAA, recognizing alpha2,3-linked sialic acids, and a novel IgY antibody recognizing 6’-sulfated sialyl Lewis X and its related non-fucosylated form, completely blocked binding of Siglec-F-Ig to mouse epithelium. Primary airway epithelial cells propagated in vitro also express Siglec-F ligands whose expression is increased by IL-4, and preliminary western blot analyses has identified several high molecular weight bands representing putative epithelial lung ligands for Siglec-8 and Siglec-F.

Conclusions: Mechanisms of human and mouse eosinophil apoptosis appear to be different via Siglec-8 and Siglec-F. High molecular weight lung epithelial-derived ligands for these two siglecs appear to contain 6’-sulfated sialyl Lewis X and/or its related non-fucosylated form. In mouse lungs, the St3gal3 sialyltransferase is required for its synthesis, but further analyses will be required to formally characterize these endogenous ligands. Antibodies or glycomimetics may someday be useful for tracking or targeting eosinophils in vivo.

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EOSINOPHILS – LONG A FOOT, BUT NOT EFFETE

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My early interests in eosinophils were engendered by their associations with helminthic parasitic infections. As a medical student I recall photocopying (onto non-durable photo-diazo paper) the cardinal papers, by Paul Beeson’ group at Oxford published in J Exp Med, demonstrating the T lymphocyte dependence of eosinophilia. My interests in eosinophils continued through my clinical training and my time in the Laboratory of Parasitic Diseases at NIH. Subsequently I entered a research lab in Boston with my focus on studying eosinophils and co-authored a review in 1979, all too presciently entitled: “The regulatory and effector roles of eosinophils in immunologic responses.” The then hypothesized regulatory roles were based on indications that eosinophils, still considered end-stage effector cells, could enzymatically inactivate mediators of immediate hypersensitivity including histamine (via histaminase) and SRS-A (cysteinyl leukotrienes) by arylsulfatase B. With purified human eosinophil arylsulfatase B, we showed that this enzyme had no role in inactivating SRS-A. Following recognition that SRS-A activity was mediated by cysteinyl leukotrienes, we established that human eosinophils were a rich source of LTC4. Thereafter, with the research of many colleagues, we delineated roles for inducible intracellular organelles, lipid bodies, in eosinophils and other leukocytes as sites of enhanced LTC4 eicosanoid formation. In addition to its function as a paracrine mediator, we identified roles for LTC4 as intracrine regulator of eosinophil cytokine secretion.

With a suspicion that eosinophils were not singularly end-stage effectors capable principally of degranulation, we began to document that eosinophils expressed cell-surface proteins, including CD4 and HLA-DR, that enabled their interactions with lymphokinones and other cells. These studies have been extended to demonstrate the capacities of eosinophils to serve as professional antigen-presenting cells.

Although early beliefs were that eosinophils, like other granulocytes, lacked the capabilities for protein synthesis, our studies of CD4 and HLA-DR expression included metabolic labeling approaches documenting de novo synthesis of these proteins by eosinophils. In an early NIH grant I even speculated that eosinophils might synthesize and release “eokines.” That speculation was roundly criticized in the summary pink sheet – a grantsmanship lesson learned. That eosinophils were a source of eokines/cytokines unfolded with the collaborative initial finding that eosinophils in lesions were sources of TGF-a and the cardinal EM immunogold finding that TNF-a was present within eosinophil granules. Subsequent studies documented that eosinophils, and their granules and secretory vesicles, contained a wide array of preformed cytokine proteins, a finding bolstered by studies of many other investigator groups, that helped identify a distinct role for eosinophils as sources of preformed cytokines.

The demonstration that eosinophil granules contained a multitude of preformed cytokines refocused attention on mechanisms of eosinophil “piece-meal” degranulation, a concept originated by our longstanding collaborator, Ann Dvorak MD. Again with the help of many expert colleagues we have been delineating mechanisms whereby granule-derived proteins may be mobilized and secreted from granules both with eosinophils and as free extracellular granules.

As the eosinophil community has so richly contributed to further delineating “The regulatory and effector roles of eosinophils in immunologic responses”, I am so grateful for collaborative contributions of fellows and staff in our lab, many of whom have continued independently to make advances to eosinophil and leukocyte biology. I am proud of their continued contributions. Likewise, collaborations with investigators in the eosinophil community have advanced our common efforts to delineate the functional capabilities and their underlying mechanisms of eosinophils.

References:
Weller (continued)


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MY ENDLESS LOVE STORY WITH EOSINOPHILS

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I first met eosinophils, during the course of studies on immune mechanisms against schistosome parasites. The use of a poorly studied animal model, the rat, and of in vitro cytotoxicity procedures, led to our demonstration of the cytotoxic function of rat eosinophils against schistosome larvae, confirmed by their in vivo protective role. Then came the wedding, and the first evidence of IgE-dependent activation of inflammatory cells, eosinophils but also of macrophages, a pioneering concept of the now called alternatively activated macrophages or aaMs. Such observations led to the identification of target antigens on the schistosomula surface, in particular a specific parasite enzyme, the P28 GST, which is, at the present time, the only schistosomiasis vaccine candidate in phase 3 clinical trials.

These observations, extended to human eosinophils, led to a major line of research aiming at the identification of eosinophil surface receptors and their mechanisms of activation. Among the most intriguing observation was the evidence that eosinophils shared with T cells receptors such as CD28 and more recently the CD3/γδ TCR, whereas we showed that eosinophils were the first non-lymphoid cell able to produce so-called Th2 cytokines (IL-5 and IL-13), then Th1 cytokines (IFNγ). This set of observations introduced a novel concept of eosinophils as immunoregulatory cells, which has been amply confirmed in several laboratories.

As in many couples, life, during all this period, has not been a long and quiet river and fructuous controversies on IgE receptor expression, or on selective degranulation process for instance, led to a better understanding of the major differences between the rat and the mouse models and led nowadays to a peaceful consensus.

More recently, the emergence of mechanisms of innate immunity has allowed the evidence of a major role for eosinophils as sentinel cells, playing in particular a role in tumor immunosurveillance, a so far neglected function. Recent work has moreover underlined the role of eosinophils as potential cells involved in the regulation of aaMs, a role that we are currently studying in the context of Inflammatory Bowel Diseases, my current project.

So, as it can be measured from this brief outline, eosinophils played a constant and central role in my professional and private life, illustrating a personal example of a very fruitful co-evolution.
‘FROM POSTDOC TO FULL-STOP’

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**Background:** In this talk I will describe my experiences of going from a PhD student to obtaining my own independent laboratory, and subsequently the Directorship of a major Australian research centre that focuses on asthma and respiratory disease. I would like to emphasize that there are many choices and roads that you can travel after a PhD or postdoctoral fellowship. Each will road will have unique challenges but there are also common approaches that will lead to success. There is also serendipity. I will discuss my pathway, career defining meetings an decisions and would welcome questions during my talk or later on going from postdoc to where you want to be.
FROM PARASITES TO ALLERGY: DESTINY STRIKES

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I dedicate this lecture to my late parents Gianfranco Levi and Maria Malnati. I am here today principally because of their education, support and love.

My love story with eosinophils started not at my birth but as a PhD student when I studied the intriguing S. mansoni. What struck me was the forever coupled female-male; how clever the parasite was to cheat the host immune system; the challenge to help underdeveloped countries; and as a new immigrant in Israel, the possibility to advance peace with Egyptians through science.

But my scientific program dealt with schistosomula antigens and not eosinophils. I didn’t give up. I went to Harvard for post-doctoral studies under Dr. Austen to investigate eosinophils and allergy, but even there I was steered to mast cells. I was happy, but eosinophils with their Greek name haunted me. I waited until I had a solid position as a senior lecturer at the Hebrew University and decided to spend my first sabbatical with Barry and Redwan to study eosinophils and allergy.

What intrigued me investigating eosinophils in allergy was and still is, whether they are (perhaps as human nature) “good or bad cells”. I think that probably depending on the microenvironment and the timing of the specific reaction, eosinophils can be either one. Therefore the challenge for the researcher is harder but more fascinating.

My proudest achievements in eosinophil research are:

1. The observation made with Redwan and Barry that they contain several preformed cytokines, which make them extremely “ready to go” immunomodulators.
2. TGF-β promotes fibrotic changes of fibroblasts and therefore are key cells in the connective tissue remodeling.
3. The expression of three activating receptors belonging to the CD2 family namely CD48, 2b4 (CD244) and Nectin-2. CD48, the most interesting, is one of the mouse asthma signature genes and is increased on eosinophils from asthmatics. Also, αCD48 antibodies abrogated OVA induced murine asthma (work done in collaboration with Marc, Fred, and my former student Ariel).
4. The expression of several inhibitory receptors, the most important being CD300a. Its activation by bispecific antibodies directed to CD300a and to CCR3 in vitro blocked eosinophils activation carried out by several agonists and in vivo abrogated a chronic model of murine asthma.
5. The existence and functional activity of the Allergic Effector Unit (AEU) consisting of the soluble and physical cross-talk between mast cells and eosinophils that takes place in vitro and in vivo, in allergic tissues.

The effect of hypoxia on the pro-angiogenic potential of eosinophils that we found to be mediated by eosinophil derived VEGF, MBP and osteopontin.

Currently we are working on the AEU focusing on CD48 and 2b4, on eosinophils and hypoxia and on the activation of the inhibitory receptors. My ultimate goal is to design better drugs for patients suffering from allergic and other diseases with a clear eosinophil involvement.

Finally, I advise young scientists to remain “forever young” as eosinophils, and as your eosinophilomaniac senior colleagues who have never lost their curiosity to ask and search.
THE EOSINOPHIL AS A TEACHER: "BASIC" LESSONS AND INSIGHTS!

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Life is an unremitting school; everything within it is a teacher, but only if we are willing to assume the posture of learners! Scientifically, I am privileged to have worked on the eosinophil since 1978 (over 33 years). My journey with this “basic research partner” has been at once exciting, educating and challenging. In my presentation, I will attempt to reflect on what I have learned from observing three major functions ascribed to the eosinophil (at least from my research work perspective). These three roles are: (1) effector, (2) tissue remodeler, and (3) immune regulator. In looking at nature and especially within the context of these three cellular functions, I have to come to realize that there are various lessons that may provide insights and be worthy of contemplation and reflection. After all, with over 33 years of association history with this attractive and intriguing cell type, I guess we are allowed a degree of eosino-philosophy, providing it is done with a willingness to learn and apply!
EOSINOPHIL PRIMING AND ACTIVATION: IT TAKES TWO TO TANGO

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The immune system faces an important dilemma. Fast and robust responses are necessary to combat invading microorganisms, but sufficient compensatory mechanisms should prevent the induction hyperactivation of the system. Such hyperresponsiveness of the immune system can cause damage to host tissues, which is typically found in chronic inflammatory lesions. Relatively much is known about the activation of innate immune mechanisms, but there is a surprising backlog in knowledge regarding the feedback control initiated by these activation mechanisms. One of the control modules involved in preventing hyperresponsiveness is controlled by a process generally referred to as priming. Knowledge regarding this mechanism, which was firstly described for eosinophils in the late eighties.

The concept of priming: A cellular response is primed by a priming agent when this agent does not evoke the response itself but amplifies this response by a heterologous stimulus. Mediators can prime certain responses and activate others.

Priming in vitro. The activation of human eosinophils with physiologically relevant stimuli such as opsonized targets is typically controlled by priming. Several priming agents have been described for eosinophils: chemotactic lipids (e.g. PAF) and chemokines (e.g. eotaxin), cytokines (e.g. GM-CSF/IL-3/IL-5), complement fragments (e.g. C5a) and pathogen associated molecular patterns (PAMP’s). Many of these priming agents have in common that they can activate responses that facilitate differentiation and homing of eosinophils to the tissues: differentiation, adhesion, transendothelial migration, and chemotaxis. On the other hand, these agents are potent primers of cytotoxicity associated events such as activation of phagocytosis, respiratory burst and degranulation.

Apart from above mentioned soluble mediators also immobilized ligands can potently prime cellular functions such as induction of survival and priming cytotoxicity associated responses. Ligation of b2-integrins clearly modulates the response of eosinophils activated by coated immunoglobulins pointing at the complex interplay between adhesion- and Fc-receptors. Eosinophils adhered to a physiologically relevant surface respond towards IL-8 with an increase in [Ca2+]i. Also survival of eosinophils in vitro is clearly modulated by adhesion.

Priming in vivo. Several lines of evidence show that priming of eosinophils occurs in vivo in patients with allergies and other chronic inflammatory diseases. These priming responses are found in the context of adhesion, transendothelial migration, chemotaxis, and activation of the respiratory burst and degranulation. Eosinophil priming might be, therefore, an interesting diagnostic tool to determine and study systemic inflammation in patients in vivo. However, all these responses need isolation of cells which makes the measurement of these responses difficult to apply in clinical practice. Apart from these practical considerations also the occurrence of isolation artifacts makes it difficult to interpret and compare different studies.

Recent developments show that priming of eosinophils can be measured in vivo without the need of isolation by application of antibodies only recognizing receptors in their active configuration such as integrins and Fc-receptors. This approach enables to study eosinophil priming in vivo in health and disease.
Impact of Endocannabinoids on Human Eosinophil Activation: Involvement of CB2 Receptors and Eicosanoids.

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Background: Endocannabinoids are endogenous lipids that activate cannabinoid receptors. The two main endocannabinoids are 2-arachidonoyl-glycerol (2-AG) and N-arachidonyl-ethanolamine (AEA). While their involvement in nociception and obesity are recognized, their role as immunomodulators remain elusive. Interestingly, they promote an intriguing profile of anti- and pro-inflammatory effect. This might be related to the arachidonyl moiety contained in their structure, arachidonic acid being the precursor of several pro- and anti-inflammatory eicosanoids such as the leukotrienes (LTs). We recently published 2-AG activated human neutrophils through a mechanism independent of cannabinoid receptor activation. This was the consequence of a rapid conversion of 2-AG into LTB4. Given eosinophils express the CB2 receptor and monoacylglycerol lipase (MAGL), which hydrolyzes 2-AG into arachidonic acid, we postulated endocannabinoids might activate eosinophils by additional mechanisms distinct from CB2 receptor activation.

Objectives: 1) to assess the impact of endocannabinoids on eosinophil functional responses; 2) to elucidate the cellular mechanisms by which endocannabinoids mediate their effects.

Results: In contrast to AEA, 2-AG rapidly induced the migration of eosinophils as well as the biosynthesis of eicosanoids (LTC4 and eoxin C4). Although the CB2 receptor was detected by RT-PCR and that the CB2 receptor antagonist AM630 inhibited the effect of 2-AG by ~50%, CB2 receptor agonists did not induce eosinophil migration. Interestingly, the effects of 2-AG on eosinophil migration and eicosanoid biosynthesis were prevented by the known MAGL inhibitors JZL-184 and MAFP, indicating that arachidonic acid or eicosanoids were likely involved in the stimulatory effect of 2-AG. The use of selective inhibitors of key eicosanoid biosynthetic pathways indicate a metabolite from the 15-lipoxygenase pathway might be involved in mediating the effect of 2-AG on eosinophil migration. However, neither 15-HETE, eoxin C4 nor 15-HETE could induce a significant eosinophil migration. Additional migration experiments were performed and showed that the combination of the CB receptor agonist CP 55,940 and arachidonic acid led to a similar migration than that observed with 2-AG. Neither lipoxin A4, 15-HETE, nor eoxin C4 mimicked the effect of arachidonic acid in this experimental model.

Conclusions: Altogether, our data indicate that 2-AG activates human eosinophils ex vivo. This activation implicates its hydrolysis into arachidonic acid (eicosanoid biosynthesis and migration), the activation of the 15-LO pathway (migration) and the activation of cannabinoid receptors (migration). The data support a pro-inflammatory role of 2-AG in the regulation of eosinophil functions.

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NEGATIVE REGULATION OF EOSINOPHIL PRODUCTION BY TLR4 LIGAND

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Background: It has long been appreciated that a drop in eosinophils is associated with acute bacterial infection while, in contrast, blood levels of monocytes and neutrophils rise. The initial eosinopenic response is believed to be secondary to margination of circulating eosinophils to blood vessel walls, but the mechanism for prolonged eosinophil depletion with bacterial infection remains undefined.

Objective: To investigate the consequence of exposure to microbial products, specifically bacterial lipopolysaccharide (LPS), on eosinophil production.

Methods: Low density bone marrow (LDBM) cells were cultured in 100ng/ml stem cell factor (Peprotech) and 100ng/ml FLT3-ligand (Peprotech) from Day 0 to Day 4. On Day 4, the media was replaced with medium containing 10ng/ml murine IL-5 (Peprotech). Media was changed every 2 days thereafter. Total cellular RNA was extracted at indicated time points. To induce endotoxemia, BALB/c mice were injected intraperitoneally with LPS. Bone marrow was collected and LDBM cells were isolated 24 hours after treatment. CFU assays were plated in methylcellulose-based medium containing either IL-5 alone or the cytokine cocktail of IL-5, GM-CSF (Peprotech) and IL-3 (Peprotech). Colonies on each plate were counted following 7-10 days and normalized to number of cells plated in each dish. Cytokine levels in culture supernatants were measured via commercially available ELISA kits. Expression of surface markers was determined by flow cytometry.

Results: IL-5 stimulation of progenitors resulted in induced expression of six TLRs with highest expression of TLR2 and TLR4 noted throughout eosinophil development. Surface expression of TLR4 was confirmed on CD34+ progenitors by FACS. Stimulation of progenitors with LPS at 0.001, 0.01, 0.1 and 1 mg/ml markedly inhibited IL-5-mediated eosinophil production by 21%, 33%, 50% and 69% respectively. Studies using an anti-IFN-g neutralizing antibody demonstrated that LPS suppression of eosinophil differentiation was independent of IFN-g. Further mechanistic analyses revealed a significant reduction (>80%) in progenitor proliferation without inducing apoptosis following LPS exposure. Finally, in vivo LPS administration specifically reduced numbers of eosinophil progenitors in the bone marrow in a dose-dependent manner.

Conclusions: Taken together, these findings propose a direct effect of LPS on eosinophil progenitors via inhibition of IL-5-induced proliferation as an explanation for eosinopenia following bacterial infections and a potentially novel therapeutic strategy for inhibiting peripheral eosinophilia in eosinophil-associated diseases.
**N-GLYCANS AND EOSINOPHIL TRAFFICKING**

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**Background:** The interaction of eosinophils and vascular endothelial cells are important early events in the sequestration of eosinophils to sites of allergic inflammation. In addition to the contribution of selectins, integrins and vascular adhesion molecules such as VCAM-1 and ICAM-1, recent studies have demonstrated an important role for glycans in regulating leukocyte trafficking (rolling, adhesion and transmigration) and their recruitment to sites of inflammation under conditions of physiologic shear stress. For instance, heparan sulfates expressed by endothelial cells regulate the trafficking of both neutrophils and eosinophils during inflammation (1,2). Likewise, galectin-3 (Gal-3) can function as a cell surface adhesion molecule by interacting with N-glycans, including those elaborated by UDP-N-acetylglucosamine:α-6-D-mannoside β1,6 N-acetylglucosaminyltransferase V (Mgat5), to promote eosinophil trafficking (3) and allergen-induced airway remodeling in mice (4). Interestingly, although Gal-3 lacks a transmembrane domain, it is expressed on the eosinophil cell surface (potentially via binding to N-glycans elaborated by Mgat-5) and can promote eosinophil rolling and adhesion on vascular endothelium under conditions of flow (3). However, the overall mechanisms by which N-glycans regulate leukocyte trafficking in the context of allergic inflammation is not known.

**Objective:** To understand the importance of Mgat5-modified N-glycans in the regulation leukocyte (eosinophil and neutrophil) trafficking and recruitment during allergic airway inflammation.

**Results:** Studies using wild type (WT) and Mgat5 deficient mice, revealed that allergen-challenged Mgat5-/- mice exhibit significantly diminished airway eosinophilic inflammation (BALF and lung tissue), lung Th2 cytokines and allergen-induced inflammatory responses including mucus secretion compared to WT mice. In contrast to the diminished eosinophilia, the allergen challenged Mgat5-/- mice developed significant neutrophilia (increased neutrophils in both BALF and lung tissue) with persistent elevated levels of proinflammatory cytokines (eg IL-17A) and increased airway hyperresponsiveness. Bone marrow derived Mgat5-/- eosinophils (isolated using methods developed by Dyer et al (5)) demonstrate reduced trafficking (rolling and adhesion) on Gal-3 and VCAM-1, associated with reduced basal [Ca^{2+}] levels as well as increased apoptosis compared to WT eosinophils, which could account for the decreased airway eosinophilia in allergen-challenged Mgat5-/- mice. In contrast, Mgat5-/- neutrophils exhibit increased adhesion to P-selectin and migration in response to KC associated with increased surface adhesion molecule and chemokine receptor expression, potentially accounting for the increased recruitment of neutrophils to sites of inflammation, including the airways.

**Conclusions:** N-glycans modified by Mgat-5 play an important role in mediating allergic inflammation by exerting a divergent affect on eosinophil vs neutrophil trafficking and their recruitment to sites of inflammation.


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EOSINOPHILS REGULATE LOCAL IMMUNITY DURING MUSCLE INFECTION BY TRICHINELLA SPIRALIS

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Background: Eosinophilia is a prominent feature of helminth infection. The parasitic nematode Trichinella spiralis establishes chronic infection in skeletal muscle. We have shown previously that in two models of eosinophil-ablated mice, T. spiralis larvae die in large numbers in the muscle and parasite death correlates with induction of local Th1 immune responses.

Objectives: We hypothesize that eosinophils regulate parasite growth and promote a Th2 immune response that preserves parasites in tissue.

Methods: For adoptive transfer experiments, eosinophils were isolated from spleen and peritonial lavage of infected IL-5 transgenic mice by either positive or negative MACS bead selection. For positive selection, eosinophils were enriched using PE-conjugated anti-Siglec-F antibody (BD) and anti-PE microbeads (Miltenyi Biotec). For negative selection, contaminating cells were labeled with PE-conjugated rat anti-mouse CD90.2, B220, NK1.1, CD11c, F4/80, 1A8, and Ly-6C antibodies (eBioscience) and anti-PE microbeads (Miltenyi Biotec). 5 × 10⁶ purified cells were injected intravenously into ΔdblGATA mice every two days between 5 and 17 days post infection (dpi). Infection of animals, leukocyte isolation from the diaphragm, histochemical staining, and immunohistochemistry were conducted as described previously (1). Staining and flow cytometry were done according to the manufacturer’s instructions (eBioscience). To characterize macrophage functional phenotypes, gene expression studies were conducted on total RNA isolated from diaphragm tissue using TRIZOL. To evaluate parasite growth, larvae were recovered by digesting minced diaphragms for 15 min at 37°C in 5mg/ml of collagenase I (Sigma Chemical Co). Parasites were straightened with warm (56°C) 70% ethanol and prepared for cytospin in 5%glycerol/70%ethanol solution. The cytospin slides were stained with HEMA-3 (Fisher Healthcare) and photographed using a BX51 microscope (Olympus). Parasite area was measured by fitting a polygon around the boundary of the larvae (Microsuite Basic Olympus software).

Results: Parasite death in eosinophil-ablated mice correlated with enhanced iNOS gene transcription in the muscle. iNOS producing cells were neutrophils and F4/80+CD11b+Ly6C+ macrophages at sites of infection. These changes were preceded by a reduced Th2 cell recruitment to infected muscle that was coincident with a lag in larval growth, suggesting compromise of the parasite’s habitat.

Conclusions: Our results suggest that eosinophils support T. spiralis growth and promote a Th2 response that favors parasite survival. These results begin to define the cellular interactions that occur at a site of chronic nematode infection in which the eosinophil functions as a pivotal regulator of immunity.

Reference:

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Hierarchical IL-5 Expression Defines a Subpopulation of Highly Differentiated “Pro-Eosinophilic” Human TH2 Cells

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Background: Each of the three TH2 cytokine genes, interleukin-4 (IL-4), IL-5, and IL-13, have very different function and contribute to eosinophilic inflammation through very different mechanisms.

Objectives: We hypothesized that TH2 heterogeneity could yield subpopulations of TH2 cells with different cytokine expression and effector functions.

Methods: Human TH2 cells were identified and/or generated using a variety of approaches, including: direct ex vivo activation with polyclonal activators and allergens, allergen specific TH2 cell lines, polyclonally activated Cytokine Secretion Assay (Miltenyi Biotec, Auburn, CA) IL-4 or IL-5 captured TH2 lines and clones, and in vitro differentiated TH2 cells. IL-4, IL-5 and IL-13 expression were simultaneously measured in human CD4 T cells using intracellular cytokine staining. Cytokine and GATA3 gene expression were measured using TaqMan polymerase chain reaction (PCR) probe/primer sets (Invitrogen, Carlsbad, CA). Chromatin immunoprecipitation (ChIP) was performed using a ChIP Assay kit (Millipore, Billerica, MA) with antibodies to GATA-3, anti-H3K4me3 or anti-H3K27me3, followed by PCR using primers specific for the IL4, IL5 and IL13 promoters.

Results: Using the multiple sources of TH2 cells noted above, we found that human TH2 cells are composed of two major subpopulations: a minority IL-5+ (IL-5+, IL-4+, IL-13+) and majority IL-5- TH2 (IL-5-, IL-4+, IL-13+) population. IL-5+ TH2 cells comprised only 20% of the TH2 cell total. Serial rounds of in vitro differentiation initially yielded IL-5- TH2 cells, but required multiple rounds of differentiation to generate IL-5+ TH2 cells. IL-5+ TH2 cells expressed less CD27 and greater PD-1 than IL-5- TH2, consistent with their being more highly differentiated, Ag exposed T cells. IL-5+ TH2 cells expressed greater IL-4, IL-13, and GATA-3, relative to IL-5- TH2 cells, both by flow cytometry and PCR. IL-5+ TH2 cells had greater GATA-3 and H3K4me3 binding to the IL5 promoter, relative to IL-5- TH2 cells, whereas there was no difference in their binding to the IL4 and IL13 promoters between the TH2 subpopulations. Conversely, H3K27me3 binding to the IL5 promoter was greater in IL-5- TH2 cells.

Conclusions: These findings demonstrate TH2 lineage heterogeneity, in which the IL5 gene is regulated in a hierarchical manner relative to other TH2 genes, with IL-5+ TH2 cells representing the most highly differentiated TH2 cell subpopulation. IL-5+ TH2 cells express greater amounts of all TH2 cytokines and had epigenetic changes consistent with greater IL5 promoter accessibility, relative to IL-5- TH2 cells. Differentiation of IL-5+ TH2 cells requires multiple rounds of antigenic stimulation, suggesting that recurrent antigen exposure may preferentially drive IL-5+ TH2 differentiation and consequent eosinophilic inflammation.

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ROLE OF IL-33 AND EOSINOPHILS IN INTESTINAL INFLAMMATION

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Background: IL-33, a recently identified cytokine, is linked to autoimmune and inflammatory diseases. Several studies define increased expression of IL-33 in the affected mucosa of patients with inflammatory bowel diseases (IBD). IL-33 can induce Th2 cytokine production and leukocyte mediator release. While eosinophils have been circumstantially associated with inflammatory bowel diseases (IBDs), very recent studies suggest IL-33 impacts eosinophil’s function by increasing eosinophil adhesiveness to extracellular matrix and inducing inflammatory mediator release in IBD.

Objectives: The goal of this project is to identify mechanisms by which IL-33 impacts eosinophil function during intestinal inflammation in murine models of IBD. We hypothesize that IL-33 increases eosinophil degranulation and intestinal inflammation.

Methods: Primary peripheral human blood eosinophils were isolated and assessed immediately or were cultured for 3 hours in the presence of IL-33 or other cytokines including IL-5, IL-1b, TNF-a or IFN-g for flow cytometric assessment of the IL-33 cognate receptor ST2. Eosinophils were also assessed for the release of the eosinophil granule protein eosinophil peroxidase (EPO) by electron microscopic techniques. Alterations in cytokine expression were analyzed by real time RT-PCR. The impact of IL-33 on eosinophil migration was measured using an in vitro colonic epithelial (T84 cell) transwell-migration assay. In vivo analysis of IL-33 and eosinophils in mouse models of inflammatory bowel disease (IBD) employed the DSS colitis model in addition to the SAMP1/SkuSlc model of Th2 cytokine associated eosinophilic ileitis. Finally human tissue from IBD tissues were stained for eosinophil specific EPX and IL-33 by immunohistochemistry.

Results: The membrane bound IL-33 receptor, ST2, was present on freshly isolated human peripheral blood eosinophils. This level was further increased on following incubation with IL-33 (100ng/ml), IL-5 as well as the IBD associated IL-1b, TNF-a or IFN-g cytokines (all 100ng/ml). IL-33 induced eosinophil degranulation as recorded by biochemical assay (>4-fold, p<0.05), in addition to electron microscopic evidence of granule depletion. Stimulation of eosinophils with IL-33 led to production of IL-8 (>6-fold, p<0.01) and IL-13 (~20-fold, p<0.001) compared to unstimulated cells. IL-33 induced increased eosinophil migration into the colonic epithelium (>3-fold). DSS colitis induced a robust increase in colonic eosinophils, ST2 transcript (>15-fold, p<0.01) and as well as IL-33 mRNA (epithelial ~30-fold, p<0.05 and whole tissue >6-fold, p<0.001). Similarly, findings were detected in another model of ileal IBD, SAMP1/SkuSlc mice. Following treatment of SAMP1/SkuSlc mice with anti-CCR3 antibody a significant decrease in ileal eosinophils (0.5-fold, p<0.001) and IL-33 was observed (0.25-fold, p<0.001). Eosinophil granule protein (EPX) and the cytokine IL-33 was observed in human IBD tissues.

Conclusions: Eosinophil activation by the pro-inflammatory cytokine IL-33 may contribute to eosinophil activation and intestinal inflammation.

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THE ROLE OF THE SMALL GTPASE RHO H IN EOSINOPHIL DEVELOPMENT AND EOSINOPHILIC DISORDERS

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Background: The small atypical GTPase RhoH is highly expressed in eosinophils and further upregulated in patients with hypereosinophilic syndrome (HES), a condition characterised by chronically elevated eosinophil levels and tissue damage with sometimes fatal outcome. The function and regulation of RhoH in eosinophils is unknown.

Objectives: To determine the role of RhoH in eosinophil function and development under normal and pathological conditions.

Methods: Eosinophils were isolated from peripheral blood of healthy donors or HES patients. Expression of RhoH in eosinophils with or without IL-5 stimulation was analysed by Western blot. HL60 clone 15 cells were differentiated into eosinophil-like cells by addition of 0.5mM butyric acid. RhoH expression was analysed by Western blot. Bone marrow was isolated from RhoH-/- or wild type mice and cultured with Flt-3L, SCF and IL-5 to induce differentiation into eosinophils (bmEos; according to Dyer K et al. JI 2008). Blood, bone marrow and bmEos were stained with Diff Quick and analysed by light microscopy or stained with fluorescently labelled antibodies and analysed by flow cytometry.

Results: We find RhoH is upregulated in some HES patients and that IL-5 upregulates RhoH in eosinophils from normal donors but not patients. Furthermore, it is upregulated during in vitro differentiation of eosinophils. Furthermore, RhoH-/- mice have elevated eosinophils levels in blood and bone marrow and ex vivo bone marrow eosinophils appear to be more mature. In vitro differentiation into eosinophils with IL-5 is also enhances in RhoH-/- bone marrow cultures.

Conclusions: The results suggest a regulatory role of RhoH in eosinophil development and function, possibly via negatively regulating IL-5 signalling.

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INHIBITION OF EOSINOPHILIC INFLAMMATION BY SUPPLEMENTATION WITH 5-HYDROXYTRYPTOPHAN, A SEROTONIN PRECURSOR

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Background: Serotonin regulates vascular, neuronal (anxiety/depression), and immune responses suggesting that dysregulation of the serotonin pathway may alter associated physiological functions. Reports indicate an association of anxiety/depression in individuals with allergy/asthma and indicate increased anxiety in allergen-challenged rodents. Reductions in serotonin synthesis during anxiety are reported to be a result of reduced synthesis of the serotonin precursor, 5-hydroxytryptophan (5-HTP). Local serotonin levels mediate a balance between serotonin binding to inhibitory and stimulatory serotonin receptors. This balance likely regulates inflammatory responses since serotonin receptors differ in affinity and are expressed by leukocytes and by endothelial cells which regulate leukocyte recruitment. Moreover, serotonin is released from inflammatory cells during allergic responses.

Objective: We hypothesize that dietary supplementation with the amino acid 5-HTP reduces allergic inflammation induced by OVA or by house dust mite.

Methods:
Diet. The diet with 0.024g 5-HTP (Diet #102561) and the control diet without 5-HTP (Diet #101591) were from Dyets, Inc. C57BL/6J female mice were started on the diets one day before the first treatment with OVA or house dust mite.

OVA or house dust mite administration in vivo. For OVA, the mice were i.p. sensitized with OVA (10 μg)/alum or saline/alum on days 1 and 8. On days 15, 18, and 20, the mice were challenged with intranasal OVA (150 μg) or saline. On day 21, the mice were analyzed for lung lavage leukocytes, cytokines, and chemokines. Plasma and lung tissue were examined for 5HTP metabolites by HPLC/ECD. Frozen lung sections were fixed and labeled with anti-serotonin to detect serotonylation or stained with H&E.

For house dust mite, mice received intratracheal challenge with Dermatophagoides pteronyssius extract (10 μg DerP1/50 μl saline, Greer Labs) 3 times per week for 3 weeks. Twenty-four hours after the last antigen challenge, tissues were collected and analyzed as described above.

In vitro cell association and migration assays with laminar flow. Endothelial cell monolayers were treated with 5HTP overnight and washed. Leukocyte adhesion at 2 minutes and transendothelial migration at 15 minutes was examined under conditions of laminar flow of 2 dynes/cm².

Results: In these two models of allergic inflammation, supplementation with 5-HTP significantly reduced lung lavage eosinophils without affecting body weight, blood eosinophils, Th1/Th2 cytokines, or chemokines. An additional novel finding was that allergen-challenge induced serotonylation (covalent binding of serotonin to proteins) in lung endothelial cells. Moreover, this endothelial serotonylation was blocked by 5-HTP supplementation. In vitro, 5-HTP-pretreatment of endothelial cells blocked TNFa-induced endothelial cell serotonylation and blocked leukocyte transendothelial migration, suggesting that 5-HTP regulates endothelial cell function during leukocyte recruitment in vitro and in vivo. Inhibition of endothelial cell 5HTP metabolism to serotonin blocked the inhibitory effects of 5HTP in vitro.

Conclusions: In summary, dietary supplementation with the readily available amino acid 5-hydroxytryptophan (5HTP) inhibits eosinophil recruitment to the lung and inhibits leukocyte transendothelial migration in vitro. This novel approach for regulation of eosinophilic inflammation identifies potential targets for intervention in allergic inflammation.

Grant Support: NIH: HL R01 AT00483, American Heart Assoc: GRNT2260905
RESLIZUMAB IN CHILDREN AND ADOLESCENTS WITH EOSINOPHILIC ESOPHAGITIS: RESULTS OF A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED STUDY

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Background: Eosinophilic esophagitis (EoE) is a disease characterized by chronic, isolated eosinophilic infiltration of the esophagus associated with difficulty swallowing, vomiting, chest pain and possible tissue remodeling and stricture formation. Results from animal studies suggest that interleukin-5 (IL-5) induces eosinophil trafficking in the esophagus.

Objective: To evaluate the effect of reslizumab, an antibody to IL-5, in children and adolescents with EoE.

Methods: Patients aged 5 to 18 yrs with vomiting, regurgitation, abdominal pain, chest pain/heartburn, and/or dysphagia of moderate or worse severity; an esophageal biopsy with ≥24 intraepithelial eosinophils per hpf (peak count); and treatment with proton pump inhibitors for ≥4 wks without symptom or histologic resolution or a negative pH probe test result were eligible. Patients were randomly assigned to receive infusions of reslizumab 1, 2, or 3 mg/kg or placebo at wks 0, 4, 8, and 12. The co-primary efficacy measures were the percentage change in peak esophageal eosinophil count based on a blinded central pathology review and change in a physician’s EoE global assessment score at week 15 (end of therapy).

Results: 226 patients (mean [SD] age: 11.9 [3.95] years) received study medication: 76.1% were boys. The median peak esophageal eosinophil count from the screening biopsies was 80 cells per hpf. The predominant symptoms were dysphagia in 43% of patients, abdominal/chest pain in 39%, and vomiting/regurgitation in 17%. Median reductions from screening to end of therapy in peak esophageal eosinophil counts were 59%, 67%, 64%, and 24% in the reslizumab 1, 2, and 3 mg/kg and placebo groups, respectively (all p<0.001 vs placebo). Median peak eosinophil counts in the treatment groups at end of therapy were 37, 27, 30, and 93 cells per hpf, respectively. All treatment groups, including the placebo group, showed improvements in the physician’s global assessment scores; the differences between the reslizumab and placebo groups were not statistically significant. The incidence of adverse events in the reslizumab groups was similar to placebo. The most common adverse events in the reslizumab groups were headache, cough, nasal congestion, and upper respiratory infection. One patient in each reslizumab group and 2 in the placebo group had serious adverse events; none were considered by the investigators to be related to study medication.

Conclusion: Reslizumab significantly reduced intraepithelial esophageal eosinophil counts in children and adolescents with EoE. Improvements in the physician’s global assessment scores were observed in all treatment groups and were not significantly different between the reslizumab and placebo groups. Reslizumab was generally well tolerated in this study.

Grant support: This study was funded by Ception, Inc., which has since been acquired by Cephalon, Inc.
THE GENERATION AND CHARACTERIZATION OF INDUCIBLE EOSINOPHIL-LESS TRANSGENIC MICE

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Background: Eosinophils are rare innate immune leukocytes best known for their destructive end-stage effector function in tissue damage associated with allergic inflammation and helminth parasitic infection. In addition to the end-stage effector role, recent evidence from human and animal study has revealed a more complex role for eosinophils in modulating immunity, inflammation and tissue remodelling.

Objective: To develop and characterize an inducible transgenic model to study the role of eosinophils by targeted ablation.

Methods: In the inducible eosinophil-less mice (iPHIL), the diphtheria toxin (DT) receptor (DTR) is expressed by eosinophil peroxidase (EPX) promoter which renders naturally DT-resistant mouse eosinophils DT-sensitive to DT administration. To determine eosinophil blood, bone marrow, and tissue kinetics at homeostatic baseline, iPHIL and wild type (C57BL/6 background) mice were injected i.p. with 15ng/g of body weight of DT on day 0 and day 1. Additional controls of saline injected iPHIL were included. Cell differential of either blood films or bone marrow preparations were performed for 10 days on slides stained with Diff Quik stain (SIEMENS), counting ≥300 cells. Thymus, uterus, femur, intestine and spleen were harvested and assessed for tissue eosinophil levels. Specifically, formalin-fixed tissues were stained with a rat anti-mouse monoclonal eosinophil major basic protein (MBP) antibody to detect eosinophil accumulation by immunohistochemistry.

Results: Administration of DT resulting in complete ablation of circulating blood eosinophils within 4 to 5 days. With these time points we were able to calculate the half life of eosinophils in circulation to be 1.2 days. The depletion of eosinophils in the blood is mirrored in the bone marrow, both of which return to baseline levels of eosinophils within 9-10 days post-injection of DT. In all tissue examined, iPHIL mice have significant decrease in MBP positive cells (i.e., eosinophils) between day 0, 5 and day 10 of the treatment suggesting tissue eosinophils have longer half life than in circulation. Additionally, eosinophils levels were unaffected in the uterus, suggesting a unique environment that enhances eosinophil survival.

Conclusion: We successfully generated a novel mouse model (iPHIL) that allows us to induce ablation of eosinophils in circulation, bone marrow, as well as in some tissues. This strain of mouse permitted the observation that eosinophils in circulation have a shorter half life than those of in tissue, particularly in the uterus. Thus, the iPHIL mouse model is an excellent tool to study the role of eosinophils in the pathogenesis of allergic inflammation, parasitic infection, as well as many other diseases.

Grant Support: This project was funded by grants from the NIH NHLBI R01 HL058723, Canadian Institutes of Health Research, The Lung Association of Alberta and NWT, and Mayo Foundation for Medical Education and Research.
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HUMAN EOSINOPHILS EXPRESS FUNCTIONAL CCR7

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Background: Several lines of evidence have demonstrated that eosinophils act as antigen-presenting cells (APCs), including exhibiting requisite trafficking to regional lymph nodes for antigen presentation. APCs and T cells both home to lymph nodes through stimulation of the chemokine receptor CCR7, facilitating interaction of APCs and T cells. Though a prior report reported CCR7 expression in an eosinophilic leukemia cell line, it has been unknown whether human eosinophils express functional CCR7.

Objective: We hypothesized that human eosinophils express CCR7 and that this CCR7 expression is functional as evidenced by assays of chemotaxis, shape change, calcium flux, and downstream ERK phosphorylation upon stimulation of eosinophils with the known ligands of CCR7, CCL19 and CCL21.

Methods: Eosinophil Purification and CCR7 Detection: Human eosinophils were purified by negative selection from healthy donors. Surface CCR7 expression was measured by flow cytometry (BD FACScan) of eosinophils primed for 2 days with IL-5 (10ng/ml). CCR7 expression was also detected by Western blots of IL-5-stimulated eosinophils.

Chemotaxis: Chemotaxis of IL-5-primed eosinophils was measured by use of a transwell system (pore size 0.5 mm) with the upper chamber loaded with 2 x 105 eosinophils and the lower chamber with chemokine-containing medium. After 1 hour of incubation, migrating eosinophils in the lower chamber were counted by flow cytometry.

Shape Change: Shape change of IL-5-primed eosinophils after stimulation with CCL19 or CCL21 was imaged using phase microscopy with an inverted microscope (Nikon TE-300) and analyzed by flow cytometry. Eosinophils were also stained for F-actin with Alexa 488-conjugated phalloidin and with Hoescht nuclear dye, followed by imaging with an Olympus BX62 upright microscope.

Calcium Flux: IL-5-primed eosinophils were loaded with fluo-4 AM. Cells were stimulated with CCL19 or CCL21 (1mg/ml) and fluorescence was recorded over time by flow cytometry.

ERK Phosphorylation: IL-5-primed eosinophils were stimulated with CCL19 or CCL21 (500ng/ml). ERK1/2 phosphorylation was assayed by Luminox assay at 1, 3, 5, 15, and 60 minutes. Additionally, ERK1/2 phosphorylation was assayed by western blot at 1, 3, and 10 minutes.

Results: IL-5-primed human eosinophils expressed CCR7, as demonstrated by both Western blots and cell surface flow cytometry. Primed eosinophils exhibited chemotaxis toward both CCL19- and CCL21 (p < 0.05 for both 200ng/ml CCL19 and CCL21 compared to vehicle). Chemotaxis toward both CCL19 and CCL21 occurred in a concentration-dependent fashion. Upon stimulation with CCL19 or CCL21, primed eosinophils demonstrated dose-dependent shape change with polarization of F-actin. IL-5-primed eosinophils evidenced demonstrable calcium influxes when stimulated with CCR7 ligands. Finally, primed eosinophils stimulated with either CCL19 or CCL21 exhibited increased phosphorylation of ERK in response to both CCR7 ligands.

Conclusions: Human eosinophils, after priming with IL-5, express the chemokine receptor CCR7. Eosinophils demonstrate a robust chemotactic response to CCL19 and CCL21, the ligands of CCR7. Primed eosinophils also exhibit shape change, calcium flux, and ERK phosphorylation when stimulated through CCR7. These findings suggest that the APC activity of eosinophils may be facilitated by the presence of functional CCR7.

Grant Support: NIH-F32AI081513 (PA); NIH-R37AI020241 and NIH-R01AI051645 (PFW)
POSTER A-2

EOSINOPHILS PROMOTE RESOLUTION OF ACUTE PERITONITIS BY PRODUCING 12/15-LIPOXGENASE-DERIVED LIPID MEDIATORS IN MICE

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Background: Acute inflammation in healthy individuals is self-limiting and has an active termination program. The mechanisms by which acute inflammation is resolved are of interest. In zymosan-induced peritonitis, we found that eosinophils are recruited to the inflamed loci during the resolution phase of acute inflammation.

Objectives: We hypothesize that eosinophils may contribute to the resolution of acute peritonitis by releasing anti-inflammatory mediators.

Methods: For acute peritonitis, male C57BL/6 mice were given i.p. injection of zymosan A (1mg). At the indicated time points, peritoneal exudates were collected by lavaging with 5 ml of sterile PBS. For determination of cellular composition, peritoneal leukocytes were stained with antibodies to either PE-conjugated anti-mouse Gr-1, FITC- or PE-Cy5-conjugated anti-mouse F4/80, or FITC-conjugated anti-mouse CCR3. Cells were analyzed for composition with a fluorescence-activated cell sorter (FACS) using FACSCalibur with data analyzed by CellQuest. For eosinophil depletion, either 5 μg of anti-mouse IL-5 antibody (TRFK-5) or its isotype control rat IgG1 (R3-34) was injected intraperitoneally twice before zymosan challenge. For LC-MS/MS analysis, a triple quadrupole linear ion trap mass spectrometer (4000QTRAP) equipped with an Acquity UPLC BEH C18 column (1.7 μm, 1.0 × 150 mm) was used. MS/MS analyses were conducted in negative ion mode, and lipid mediators were quantified by multiple reaction monitoring. Calibration curves and LC retention times for each compounds were established with synthetic standards. Eosinophils were isolated from transgenic mice carrying the mouse IL-5 gene ligated with a metallothionein promoter (kindly provided by Dr. Kiyoshi Takatsu). Eosinophils were isolated from peritoneal lavages of IL-5 transgenic mice that were injected intraperitoneally with cadmium-containing saline (200 mg/ml) 48 hours before harvesting. Cell suspension (3 mL) was layered on top of 4 mL Percoll (60%; 1.084 g/mL). After centrifugation (30 min, 1,500 g, room temperature), the leukocyte layer was recovered and washed twice with PBS. 12/15-LOX deficient eosinophils were isolated from 12/15-LOX deficient mice crossed with IL-5 transgenic mice.

Results: In vivo depletion of eosinophils caused a resolution deficit, namely impaired lymphatic drainage with reduced appearance of phagocytes carrying engulfed zymosan in the draining lymph node, and sustained numbers of polymorphonuclear leukocytes in inflamed tissues. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics of the resolving exudates revealed that locally activated eosinophils produced 12/15-lipoxgenase-derived lipid mediators including protectin D1 (PD1) from docosahexaenoic acid. The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the administration of PD1. Eosinophils deficient in 12/15-lipoxgenase were unable to rescue the resolution phenotype.

Conclusions: The present results indicate that eosinophils and eosinophil-derived lipid mediators including PD1 have a role in promoting the resolution of acute inflammation, expanding the roles of eosinophils in host defense and resolution.


Grant Support: This work is funded by PRESTO, Japan Science and Technology Agency.
EOSINOPHILS AS NOVEL CELL SOURCE OF PROSTAGLANDIN D2: AUTOCRINE ACTIVITY AND ALLERGY-DRIVEN SYNTHESIS

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Background: Prostaglandin (PG) D2 is a key mediator of allergic inflammatory diseases, like asthma. This eicosanoid is a cyclooxygenase product synthesized mainly by mast cells which constitutively express high levels of the terminal enzyme involved in PGD2 synthesis, the hematopoietic PGD synthase (h-PGDS).

Objectives: Here, we investigated whether eosinophils are also capable to synthesize and, therefore, supply biologically active PGD2 when properly stimulated.

Methods: Potential PGD2 synthesis was considered within (i) human eosinophils isolated from peripheral blood of health volunteers with a negative selection kit (as approved by 052/09 CEP UFRJ/HUCFF); mouse eosinophils differentiated in vitro from mouse bone marrow cells (1); and (iii) eosinophils recruited to inflammatory sites of in vivo mouse model of allergy (as approved by CEUA/FIOCRUZ L002/08). Biologically activity of eosinophil-derived PGD2 was studied by employing inhibitors of PGD2 synthesis and activity.

Results: Cytoplasmic constitutive expression of H-PGDS was found within non-stimulated human circulating eosinophils of healthy donors. Acute stimulation of human eosinophils with A23187 (0.1 – 5 μM) evoked PGD2 synthesis, which was located at the eosinophil nuclear envelope and was inhibited by pre-treatment with HQL-79 (10 μM), a specific inhibitor of H-PGDS. Pre-stimulation of human eosinophils with arachidonic acid (AA; 10 μM) or human eotaxin (100 ng/mL) also enhanced HQL-79-sensitive PGD2 synthesis, which, by acting on membrane-expressed specific receptors (DP1 and DP2), displayed an autocrine/paracrine ability to activate lipid body biogenesis and leukotriene (LT)C4 synthesis, hallmark events of eosinophil activation. Similar to human eosinophils, in vitro-differentiated mouse eosinophils also synthesized PGD2 within lipid bodies in response to AA stimulation. Infiltrating eosinophils found at the inflammatory site of the allergic reaction were identified as a PGD2-synthesizing cell population.

Conclusions: Our findings reveal that eosinophils are indeed able to synthesize and secrete PGD2, hence representing during allergic inflammation an extra cell source of PGD2, which functions as an autocrine signal for eosinophil activation.


Grant Support: From Brazilian agencies FIOCRUZ, CAPES, CNPq and FAPERJ, as well as National Institutes of Health (Bethesda, MD) grants to P.F.W (AI020241, AI051645 and AI022571).
Poster A-4

Polymorphisms in the ECP and EPX/EDN Genes in the IBD4 Locus: Gender and Age Related Associations with Inflammatory Bowel Disease and Cancer Development

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Background: A role of eosinophil granulocytes in inflammatory bowel disease (IBD) has been suggested. The intraluminal release of eosinophil derived cytotoxic proteins is increased in patients with ulcerative colitis (UC) [1] and larger number of activated eosinophils, identified as CD44-high has been reported. This was seen in both UC and Crohn’s disease (CD), but was more pronounced in quiescent as opposed to active UC [2]. Polymorphisms in the eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN) and the eosinophil cationic protein (ECP)-genes affect the eosinophil content of EPX and ECP and the cytotoxic activity of ECP. Furthermore both EPX and ECP have been associated with different types of cancer.

Objective: To study the impact of genetic variation in the EPX and ECP genes and their associations to IBD. The hypothesis was that alterations in the cytotoxic activities of ECP and/or the altered expression of EPX might affect different features of IBD and also affect the propensity to acquire IBD.

Methods: DNA was extracted from whole blood of 587 patients with Crohn’s disease (CD), 592 with UC and 300 healthy subjects. Diagnoses of CD and UC were based on clinical, histological and endoscopic findings, according to standardized criteria. The EPX405 (G>C, rs2013109), ECP434 (G>C, rs2073342) and ECP562 (G>C, rs2233860) gene polymorphisms were analysed, by the 5´ nuclease allelic discrimination assay. The eosinophil contents of EPX and ECP were analysed with ELISA and FEIA, respectively.

Results: Females with CD and an age at diagnosis of >45y with CD have a higher prevalence of the ECP434 and 562 GC-genotypes compared to a healthy population (range p=0.043 to p=0.009) who had more ECP434 and 562 GG-genotype. Males diagnosed with UC at an age of >45y have a higher proportion of the EPX405, ECP434 and ECP562 GC-genotypes compared to a healthy population (range p=0.05 to p=0.009). The age of disease onset was linked to haplotypes in females with CD (p=0.003) and males with UC (p=0.0009). The EPX405 genotype was linked to the protein content of EPX (p=0.009) and in a reverse fashion to ECP (p=0.035). The relative risk for UC patients with EPX434 or ECP562-GC/CC genotypes to develop dysplasia/cancer was 2.5 (1.2-5.4, 95% CI) for ECP434 and 2.5 (1.1-5.4, 95% CI) as compared to those carrying the GG-genotypes.

Conclusion: The present study has identified an age and gender related difference between patients with CD and UC and polymorphisms in the EPX and ECP genes, and also linked the EPX405 polymorphism to the eosinophil content of the proteins in patients with IBD. These novel findings suggest essential roles of ECP and EPX in the disease processes of Crohn’s Disease and Ulcerative Colitis.

References:

ANTIMICROBIAL RIBONUCLEASES: GENETIC DIVERSITY AND NOVEL STRUCTURE

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Background: Members of the vertebrate ribonuclease A family are secretory enzymes with prominent roles in promoting host defense. RNase 2 (EDN) and RNase 3 (ECP) are two of the major secretory proteins of eosinophils, with significant sequence homology (67%) to one another, and antiviral and antibacterial activity, respectively. EDN and ECP are highly divergent among non-human primates, but display only limited nucleotide sequence divergence within the human species [1]. Likewise, RNases 7 and 8 are another set of paired RNase A family genes, with significant sequence homology (78%) to one another. RNase 7, similar to ECP, is highly cationic and has substantial antibacterial activity. While the physiological function of RNase 8 is unknown, its evolution among non-human primates has generated “functional” pseudogenes, ie... disruptions to either the characteristic cysteine structure or catalytic site [2].

Objectives: We intend to assess the genetic diversity of RNases 7 and 8 within the human population and compare it to that determined previously for RNase 2 (EDN) and RNase 3 (ECP). We will also evaluate expression patterns and potential function for RNase 8.

Methods: Genomic DNA was obtained from the Coriell (Camden, NJ). Platinum Taq proofreading polymerase (Invitrogen) and gene-specific primers were used to amplify RNase 7 and 8 genes which were sequenced in both directions. Human and fetal tissue cDNA panels (Clontech) were screened using TaqMan universal PCR master mix, RNase 8 specific primer/probe sets, and ABI 7500 Real Time PCR system. Human spleen marathon RACE-ready cDNA (Clontech) was amplified using the A2 polymerase kit (Clontech) and an oligonucleotide encompassing the RNase 8 gene.

Results: Our evaluation of RNase 7 included 92 alleles from 46 individuals, and a total of 471 nucleotides. We detected 3 single nucleotide polymorphisms (SNPs) and a calculated nucleotide sequence diversity of (n = 0.00073 ± 0.00022) which is unremarkable for protein coding genes; this is similar to what we observed previously for both RNase 2 (EDN) and RNase 3 (ECP) [1]. Interestingly, despite evidence for substantial diversity among non-human primates, we detected only 2 SNPs within the coding sequence of RNase 8. Our analysis of the RNase 8 coding sequence included 54 alleles from 27 individuals, and a total of 462 nucleotides; the nucleotide sequence diversity was likewise unremarkable (n = 0.00122 ± 0.00009). Further analysis revealed predominant expression of RNase 8 in the spleen, lung, and testis, and a previously unrecognized amino terminal extension, present in RNase 8 transcripts, that presents the possibility that RNase 8 may be membrane bound.

Conclusions: We have shown that there is minimal diversity among RNase 7 and 8 alleles in the human population, similar to what we observed previously for human EDN and ECP. We have also elucidated the sequence of the RNase 8 transcript and its expression in various tissues. The RNase 8 transcript suggests potentially novel structure and physiological function.

References:

Grant Support: This work was supported by NIAID DIR funding AI000941 and AI000942.
**POSTER A-6**

**EOSINOPHILS RELEASE IL-1 BETA AND INCREASE IL-17 EXPRESSION BY ACTIVATED CD4+ T LYMPHOCYTES**

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**Background:** Eosinophils express a variety of cytokines by which they could affect other inflammatory cells; however, whether human eosinophils directly influence T lymphocyte differentiation and function remains largely unknown.

**Objectives:** Eosinophils release IL-1beta, IL-6 and TGF-beta1, which are known to regulate Th17 and T-regulatory functions in different models. We hypothesized that eosinophils affect IL-17A and FOXP-3 expressions by CD4+ T lymphocytes through the release of these cytokines.

**Methods:** Heparinized blood was centrifuged over percoll, RBCs were lysed and eosinophils were negatively selected from the granulocyte pellet by immunomagnetic beads (anti-CD16, -14, -3). CD4+ T cells present in the mononuclear fraction were negatively selected by a cocktail of antibodies (Miltinyl). CD4+ T cells were primed with anti-CD3 (1μg/ml) plus anti-CD28 (1μg/ml) for 2 days and reactivated with anti-CD3 on the 4th day in the presence of eosinophils or supernatant fluid from eosinophils. Gene expression was measured by real-time PCR after 5 hr co-culture. Protein levels were determined by ELISA after 48 hr co-culture.

**Results:** Eosinophils increased IL-17 mRNA and protein in primed CD4+ T cells by 2-5 fold. In contrast, the expression by unprimed CD4+ T cells was not regulated by eosinophils. Conversely to IL-17, FOXP3 expression by primed CD4+ T cells was consistently reduced by co-culture with eosinophils. Mechanistically, we found eosinophils express and release IL-1beta when maintained for 3 days with a low dose of GM-CSF. IL-1beta levels in eosinophil supernatant fluids highly correlated with eosinophil supernate activity to increase IL-17 expression by CD4+ T cells (r=0.96, p<0.001, n=7). In addition, IL-17 expression was blocked with a neutralizing antibody directed against IL-1beta.

**Conclusion:** Eosinophils enhance the production of the pro-inflammatory cytokine IL-17 via IL-1 beta, and reduce FOXP3 expression by CD4+ T cells. The loss of function of T-regulatory cells, which strictly depend on FOXP-3 expression could be associated with a damaging type-17 response in association with eosinophilic inflammation.

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POSTER A-7
FLOW CYTOMETRIC METHOD FOR QUANTITATIVE IDENTIFICATION OF EOSINOPHILS IN MOUSE TISSUES

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Background: Eosinophils are tissue granulocytes with important roles in the pathogenesis of helminth infections and allergic and hypersensitivity reactions. As such it is important to develop efficient, clear and quantitative methods for identifying these cells in tissue.

Methods: Single cell suspensions were obtained from bone marrow, spleen and lung of wild-type naïve mice, mice sensitized and challenged with ovalbumin and interleukin-5 (IL-5)Tg mice. Cells from the different tissues were stained with surface markers commonly used to identify eosinophils by flow cytometry. Cytospins where also made from the cell suspensions and these were stained with Diff-quik in order to compare the flow cytometry results with manual counting of eosinophils.

Results: We found that detection with three markers, CD45+, CD11c- and Siglec F+ provided the best overall quantitative identification of eosinophils by flow cytometry in all tissues and at all degrees of eosinophilia. In spleen and bone marrow, Siglec F was the best single marker of eosinophils; however, in the lung it was necessary to differentiate between Siglec F+ alveolar macrophages and eosinophils; this was accomplished by negative selection with an antibody directed against CD11c. We observed very high correlation between CD45+CD11c-SiglecF+ three-marker flow cytometric analysis and visual inspection of eosinophils (r²=0.98 – 0.99) in all tissues examined.

Conclusions: We show that a flow cytometry method utilizing antibodies directed against CD45, CD11c and Siglec F allows for the quantitative identification of eosinophils in bone marrow, spleen and lung cell preparations and that this method is equivalent to visual inspection at minimal and at high levels of eosinophil infiltration.

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FUNCTION OF SECRETED PHOSPHOLIPASE A2 GROUP X IN ENDOGENOUS EOSINOPHILS
LEUKOTRIENE SYNTHESIS

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Background: Secreted phospholipase A2 group X (sPLA2-X) has recently been identified in the airways of patients with asthma and may participate in cysteinyl leukotriene (CysLTs, C4, D4, and E4) synthesis (1,2). We recently demonstrated that exogenous sPLA2-X caused marked arachidonic acid (AA) release and a rapid onset of CysLT synthesis in human eosinophils that was blocked by a selective sPLA2-X inhibitor, but that CysLT formation was predominantly mediated through activation of cytosolic PLA2a (cPLA2a) by the p38 and c-Jun N-terminal kinase (JNK) kinase cascades (3).

Objective: To determine whether sPLA2-X is expressed in eosinophils, and if endogenous sPLA2-X participates in eosinophil CysLT synthesis.

Methods: Peripheral blood eosinophils were obtained from volunteers with a physician diagnosis of asthma and/or allergy and ≥ 1.2 x 10⁵ eosinophil/ml of peripheral blood. Western blots of eosinophil lysates we conducted using a rabbit polyclonal anti-sPLA2-X antibody. The expression of sPLA2-X in eosinophils was assessed by qPCR. Confocal microscopy was conducted using rabbit polyclonal anti-sPLA2-X, as well as markers for golgi (NBD C6 Ceramide), endoplasmic reticulum (PDI), and granules (MBP) to co-localize with sPLA2-X. CysLT formation and AA release was activated by N-formyl-methionyl-leucyl-phenylalanine (fMLP) treatment of eosinophils. For AA release, eosinophils were incubated for 24 h with [3H]AA (0.1 mCi/well) prior to activation. An ELISA assay measured the CysLT levels in the cell culture supernatant. Because human eosinophils contain sPLA2 group IIA (sPLA2-IIA) (4) we used a sPLA2 inhibitor, known as ROC-0929, that is selective for sPLA2-X and does not inhibit other mammalian sPLA2s at nanomolar concentrations (5). We also used a control compound, known as ROC-0428, that differs from ROC-0929 by one methyl group and is essentially devoid of sPLA2 inhibition. Inhibition of cPLA2a was conducted with Pyr-2 (pyrrophenone) and Wyeth-2 (gifipladib).

Results: Human eosinophils from patients with asthma and/or allergy expressed sPLA2-X by qPCR. Western blots demonstrated a band for sPLA2-X with a slight gel shift in eosinophil lysates suggesting that the protein has the propeptide or is glycosylated. Intracellular sPLA2-X was readily visualized by confocal microscopy demonstrating that sPLA2-X has strong co-localization to the granules, and is also found in the endoplasmic reticulum, golgi and apparent lipid bodies. In response to fMLP, the release of AA was attenuated by about 50% by the selective sPLA2-X inhibitor. CysLT formation induced by fMLP was inhibited in a dose dependent manner by the selective sPLA2-X inhibitor with about 80% inhibition at 100 nM concentration of the inhibitor. No inhibition of CysLT synthesis was observed with control inhibitor. Both of the cPLA2a inhibitors also attenuated AA release and CysLT formation initiated by fMLP.

Conclusions: The results indicate that human eosinophils express sPLA2-X within the secretory compartment, and that sPLA2-X plays a significant role in endogenous CysLT formation in these cells. Since sPLA2s are upregulated at sites of inflammation, these results imply that CysLT formation by eosinophils at sites of inflammation may be mediated by sPLA2-X.

References:

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POSTER A-9

EPO ENGAGES THE HER2 RECEPTOR VIA N-LINKED GLYCOSYLATION AND INDUCES B1 INTEGRIN CLUSTERING WITH DOWNSTREAM CONSEQUENCES IN TERMS OF CELL SIGNALLING

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Background:
Eosinophil granule proteins, such as eosinophil peroxidase (EPO) are highly toxic at high concentration. However at non-cytotoxic concentrations, they have been implicated in cell and tissue remodeling, such as growth factor expression in airway epithelial cells. The Epidermal Growth Factor Receptor (EGFR/HER) family consists of four heavily glycosylated, single-chain modular cell surface glycoproteins that interact with each other. There is no known ligand for HER2, which acts as a co-receptor, forming heterodimers with other EGFR family members. One of the consequences of HER family activation can be activation of integrins. Integrins make many important direct or indirect interactions with transmembrane signalling proteins, including growth factor receptor families. Integrin activation also modifies the cytoskeleton. As a result, integrins affect cell characteristics including proliferation, survival/apoptosis, shape, polarity, motility, gene expression and differentiation. Both integrins and growth factor receptors can facilitate activation of ERK and FAK. Previous work in our lab that led to this study includes a proteomic screen in the IMR32 nerve cell line. This showed that EPO induced phosphorylation of HER2 at the Y1248 autophosphorylation site and FAK at the Y397 autophosphorylation site. This resulted in HER2 and FAK dependent ERK activation and consequent loss of the cyclin-dependent kinase p27kip from the nucleus and upregulation of the cell proliferation marker Ki67 [1].

Objectives: We hypothesised that EPO interacts with and activates the HER2 receptor via N-linked glycosylated residues, with consequences including b1 integrin activation and downstream signalling in the normal human bronchial epithelial cell line, 16HBE14o.

Methods: Methods used include culturing of the 16HBE14o cell line, treatment with 4μg/ml EPO at various time points, and pretreatment with various inhibitors, including AG825, inhibitor of tyrosine kinase activity of HER2; anti-CD29, inhibitor of b1 integrin; PF573228, inhibitor of FAK activation; or PNGaseF, inhibitor of N-linked glycosylation. Cells were harvested in TRI Reagent for RNA Isolation and subsequent qRT-PCR analysis, or in intracellular lysis buffer (100mM KCl, 3mM NaCl, 3.5mM MgCl2, 10mM HEPES, pH 7.4) containing protease and phosphatase inhibitors and 1X Triton, for immunoprecipitation and/or Western Blot analysis. Confocal Microscopy was carried out on cells cultured on coverslips and stained with appropriate labelled antibodies.

Results: Studies showed that EPO engaged the HER2 receptor in an N-linked glycosylation-dependent mechanism, resulting in phosphorylation of the receptor. EPO-induced phosphorylation of HER2 resulted in increased expression of the HER2 receptor and in activation of b1 integrin. Together these two led to the downstream phosphorylation of FAK and ERK. A functional consequence of this EPO-HER2 receptor mediated signalling was an induction of the mucin gene MUC4.

Conclusions: Our results indicate that HER2 is a ligand for EPO and through this EPO could contribute to cell and tissue remodelling and repair, but also to the abnormal cell proliferation seen in asthma and cancer.

References:

Grant Support: We recognise grant support from The Wellcome Trust.
POSTER A-10

REGULATION OF INFLAMMATORY MEDIATOR RELEASE FROM EOSINOPHILS: ROLE OF RAB27A IN EXOCYTOSIS AND ALLERGIC ASTHMA

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Background: Inflammatory mediator release from eosinophils contributes to allergic symptoms by causing airway hyperresponsiveness, edema and tissue inflammation. The release of eosinophil mediators may be regulated by the Rab -related guanosine triphosphatase (GTPase), Rab27a. Rab27a is critical for vesicle trafficking in melanosomes and several leukocyte subsets. Our earlier studies (1) indicated that Rab27a mRNA and protein are expressed in human eosinophils and its activated form (GTP bound) was up-regulated upon stimulation. Here we determined the potential regulation of eosinophil mediator release and allergic asthma by Rab27a.

Methods: We isolated eosinophils from IL-5 transgenic (IL-5/WT) and IL-5 transgenic crossed with Rab27a-deficient mice (IL-5/Ashen) and compared their ability to release eosinophil peroxidase (EPO) in response to platelet-activating factor (PAF) and ionomycin in vitro. We also compared the EPO release from IL-5/WT and IL-5/Ashen eosinophils ex vivo and in vivo utilizing IL-5/hE2/EPO-/- transgenic mice, which better represent human asthma patients and their characteristics such as eosinophil degranulation (2). An ex vivo study was performed by co-incubating IL-5/WT and IL-5/Ashen eosinophils with bronchoalveolar lavage (BAL) fluid from IL-5/hE2/EPO-/- mice. In addition, in an in vivo study, IL-5/Ashen eosinophils were intratracheally instilled into the lungs of IL-5/hE2/EPO-/- mice. EPO release was measured in bronchoalveolar lavage (BAL) fluid using a novel and validated EPO ELISA developed in our lab. Finally, in an in vivo study, IL-5/Ashen eosinophils were intratracheally instilled into the lungs of IL-5/hE2/EPO-/- mice. EPO release was measured in bronchoalveolar lavage (BAL) fluid using a novel and validated EPO ELISA developed in our lab. Finally, in an in vivo study, IL-5/Ashen eosinophils were intratracheally instilled into the lungs of IL-5/hE2/EPO-/- mice. EPO release was measured in bronchoalveolar lavage (BAL) fluid using a novel and validated EPO ELISA developed in our lab.

Results: Eosinophils isolated from IL-5/WT and IL-5 transgenic crossed with Rab27a-deficient mice (IL-5/Ashen) showed a significant reduction in EPO release compared with IL-5/WT following stimulation with PAF and ionomycin (n ≥ 5 ~ 10, p < 0.001). In vivo and ex vivo studies also showed that IL-5/ Ashen eosinophils released less EPO than IL-5/WT eosinophils (n = 4, p < 0.001). Finally, in OVA-sensitized WT and Ashen mice, saline-challenged WT and Ashen exhibited similar responses, whereas OVA-treated Ashen showed reduced airway hyperresponsiveness than OVA-treated WT (n ≥ ≥ 6 ~ 10, p < 0.05).

Conclusions: Our findings suggest that Rab27a regulates an important transport mechanism in eosinophil granule and vesicle exocytosis. Inhibition of eosinophil degranulation was partial in Rab27a-deficient mice, suggesting that compensatory signaling mechanisms may maintain granule-derived eosinophil mediator release. Rab27a deficient eosinophils from Ashen mice demonstrated reduced degranulation in vitro and in the lungs of the mice model of asthma. This in turn affected the airway hyperresponsiveness in mice. Overall, these findings suggest that Rab27a GTPase may be an important molecular regulator in eosinophil-related allergy and asthmatic disease.

References:

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**A ROLE FOR CYSTATIN F IN EOSINOPHIL BIOLOGY**

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**Background:** The papain-like cysteine protease cathepsins are abundantly expressed lysosomal proteases with many and varied physiological roles, including the digestion of protein antigens for presentation by Class II MHC molecules, bone remodeling, and activation of effector proteins during lysosome-mediated apoptosis. In order to prevent uncontrolled proteolysis and its sequelae, tight regulation of such protease activity is necessary. The cystatin superfamily of endogenous inhibitors represents one important mechanism by which this is achieved. The cystatins are small, tight-binding cysteine protease inhibitors that are ubiquitously expressed in mammalian tissues. Unique among this family, cystatin F is an unusual example of an endogenous protease inhibitor that is itself activated by proteolysis, suggesting that it might attenuate excessive protease activity. Its expression is restricted to cells of the immune system, in particular those that use secretory lysosomes to mediate effector function, such as cytotoxic CD8 T cells, natural killer (NK) cells and neutrophils. A major target of monomeric cystatin F in immune cells is cathepsin C, which in turn activates several serine protease zymogens including granzymes A & B, neutrophil elastase and mast cell chymase [1]. However, to date no biological function has been confirmed for cystatin F in controlling the regulation of these or other activities. We recently generated cystatin F null mice to assess its function in vivo.

**Objectives:** Given the highly selective expression of cystatin F by cells that express toxic effector proteins within secretory granules, we sought to investigate its expression and function in eosinophils.

**Methods:** Mature eosinophils were isolated from bone marrow and peripheral tissues of naïve cystatin F null or wild-type mice and analysed by FACS and immunofluorescence. Bone marrow eosinophils were cultured as described [2]. Mixed wild-type / cystatin F null radiation chimerae were generated by standard methods.

**Results:** We show for the first time that cystatin F is abundantly expressed in wild-type eosinophils. Remarkably, eosinophils were present in dramatically reduced numbers in naïve cystatin F null mice and displayed reduced granularity and longevity compared to their wild type counterparts, although eosinophil progenitors were unaffected. Using in vitro eosinophil culture and radiation chimerae we confirmed that the requirement for cystatin F was cell autonomous, and that the null phenotype could be rescued by restoration of cystatin F expression.

**Conclusions:** We have demonstrated that cystatin F is required for eosinophil survival both in vitro and in vivo. This is the first demonstration of a specific, non-redundant requirement for cystatin F in vivo and indicates the importance of protease regulators in granulocyte biology. The data are consistent with a protective role for cystatin F in restraining the activity of toxic granule proteins, and we are currently working to identify the relevant targets of cystatin F in eosinophils.

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**References:**
**Poster A-12**

**Expression and Functional Roles of the Purinergic Receptor P2Y12 in Human Eosinophils**

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**Background:** Identification of new target molecules through which eosinophils activate and secrete their stored proteins may be highly significant for our understanding about allergic inflammation and host immune responses to parasites, as well as reveal new therapeutic targets for the control of eosinophilic disorders. We have recently reported the expression of the purinergic P2Y12 receptor (P2Y12R) in human eosinophils [1], however its functional roles and contributions to eosinophilic inflammation remains to be elucidated.

**Objective:** We evaluated the expression and functional roles of the purinergic P2Y12R in isolated human eosinophils.

**Methods:** We isolated eosinophils from blood of healthy donors by negative immunomagnetic selection. P2Y12R protein expression, eosinophil apoptosis and shape change were evaluated by flow cytometry. Eosinophil P2Y12R mRNA expression was evaluated by RT-PCR. Eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) measurements were assessed by colorimetric assays.

**Results:** P2Y12R protein and mRNA expression on isolated human eosinophils were confirmed using different antibodies and primers, respectively. After ADP stimulation, eosinophils secreted ECP and EPO as measured in the supernatants. Eosinophil EPO secretion in response to ADP was differentially inhibited by the blockade of P2Y12 and P2Y1 receptors, both known to be responsive to ADP and expressed on eosinophils. Furthermore, eosinophil treatment with a P2Y12R antagonist inhibited eotaxin-induced eosinophil shape change. In addition, eosinophil treatment with ADP did not induce or prevent eosinophil apoptosis after 72 h of culture in the presence of IL-5 (30 ng/ml).

**Conclusion:** We have shown that human eosinophils express the P2Y12R which activation has important functional roles in eliciting eosinophil EPO and ECP secretion and in mediating the eosinophil shape change induced by eotaxin.


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**POSTER A-13**

**EOSINOPHILS MODULATE HELPER T CELL FUNCTION VIA IDO, GLUTAMATE AND NMDA**

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**Background:**
A role for the IFN-γ-inducible rate-limiting enzyme, indoleamine 2,3 dioxygenase (IDO) in the oxidative catabolism of tryptophan has been suggested. IDO-mediated breakdown of tryptophan generates kynurenines that are biologically active on neurons. We have previously shown that eosinophils constitutively express IDO, which in turn may regulate Th1-Th2 balance in allergic diseases. Recent reports showed positive correlations of IDO activity with Th2 immune responses including atopic asthma. In addition to kynurenines, glutamate is the most common neurotransmitter associated with excitatory terminals in the central nervous system. However, prolonged and excessive release of glutamate, leading to continuous stimulation of neurons in the brain, is associated with neuronal death (excitotoxicity) in amyotrophic lateral sclerosis, HIV dementia and other neurodegenerative conditions. This glutamate-dependent cell death mechanism has recently been recognized as a potential regulator of T cell function and apoptosis. We focused on the potential role of eosinophils in the modulation of T cell function in allergy via IDO, kynurenines and glutamate.

**Objectives:**
To investigate how eosinophils contribute to modulate T cell function in IDO- and glutamate-dependent manner.

**Methods:**
Eosinophils and naïve CD4+ T cells were separated from PBMCs and differentiated to Th1 and Th2 using established methods. Expression of glutamate receptors and transporters was detected using RT-PCR, quantitative real-time PCR, Western blotting and flow cytometry. Glutamate release was measured through sequential measurements in glutamate-free medium following overnight culture of eosinophils. T cells proliferation and apoptosis was detected by CSFE- and Annexin V-staining, respectively. Ca2+ flux was measured by Fluo-3 and Fura Red.

**Results:**
Detected constitutive expression of the xCT system (Xc glutamate/cystine transporter) in human eosinophils led to continuous release of glutamate by eosinophils. Also, we found expression of NMDA (N-methyl-D-aspartate) receptors (NMDA-R) on CD4+ T cells (eosinophils did not express NMDA-R). The latter are stimulated by glutamate and kynurenines. Apoptotic and anti-proliferative effects of NMDA-R activation were exerted on Th1, but not Th2. The same was true for Ca2+ flux; both of these effects were significantly inhibited with the NMDA-R specific competitive inhibitor, MK-801.

**Conclusion:**
This study suggests that eosinophils may be a source of, and play an important role in glutamate- and kynurenines-mediated signaling. This may occur through the constitutive xCT system and IDO activation. Our data also showed that, like neurons, T cells appear to respond to glutamate and kynurenines through NMDA-R. Thus, NMDA-R expressed on helper T cells may promote Th2 polarization allergic disease, a feature that may be influenced through the eosinophil.

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**POSTER A-14**  
**MECHANISM OF EOSINOPHIL PRIMING: PKCβII-MEDIATED ENHANCEMENT OF EOSINOPHIL CHEMOTAXIS AND DEGRANULATION**

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**Background:** The transition of quiescent eosinophils to an activated state by an agonist-dependent induction of an eosinophil cell function, such as degranulation or chemotaxis, is preceded by priming. Among the gaps in our understanding of how eosinophils function is the mechanism by which the primed or activated state of these cells is achieved. Using a proteomics approach, we identified activation of PKCβIII and have characterized its interaction with the GM-CSF receptor and actin-bundling L-plastin [1].

**Objectives:** We hypothesized that GM-CSF mediated eosinophil priming may involve the signal transduction pathway for cytoskeletal reorganization and integrin activation with an involvement of the GM-CSF receptor-interacting PKCβIII kinase and L-plastin.

**Methods:** Peripheral blood eosinophils were isolated as described [1] and primed with rGM-CSF (1 ng/ml) for 1-2 h prior to assaying chemotaxis and degranulation. Priming for chemotaxis was then measured using Boyden chambers with eotaxin at concentrations of 5 and 20 nM. Priming for degranulation was assessed by detection of extracellular ECP and EPX in the medium of eotaxin stimulated cells. Control and GM-CSF stimulated eosinophils (1 ng/ml for 1 h) were kept in 12-well culture plates at a density of 106 cells/ml of RPMI 1640 supplemented with 2% FBS and treated with increasing concentration of eotaxin (5, 10, 20 and 40 ng/ml) for 2 h. The involvement of PKCβII in priming was evaluated upon inhibition of PKCβII with 4,5-bis (4-fluoroanilino)phtalimide or after treatment of eosinophils with specific siRNA.

**Results:** Phosphoproteomic analysis demonstrated the upregulation of PKCβIII activity and interaction of PKCβIII with the GM-CSF receptor and actin-bundling L-plastin. Inhibition of PKCβIII with 4,5-bis-(4-fluoroanilino)phtalimide or PKCβIII specific siRNA blocked GM-CSF induced phosphorylation of L-plastin on serine 5. Flow cytometric analysis showed an upregulation of αMβ2 integrin which was sensitive to PKCβII inhibition. In a chemotaxis assay, GM-CSF treatment allowed eosinophils to respond to lower concentrations of eotaxin which was abrogated upon PKCβII inhibition. Similarly, inhibition of PKCβIII blocked GM-CSF induced priming for degranulation as determined by release of ECP and EPX in response to eotaxin. Importantly, eosinophil stimulation with a synthetic L-plastin peptide (residues 2-19) phosphorylated on Ser5 upregulated αMβ2 integrin expression and increased eosinophil migration in response to eotaxin independent of GM-CSF stimulation. These results indicated that phosphorylated L-plastin was both essential and sufficient to propagate the upregulation of αMβ2 integrin and priming for chemotaxis and degranulation.

**Conclusions:** Our results establish a causative role for PKCβII and L-plastin in linking GM-CSF-induced eosinophil priming for chemotaxis and degranulation to signaling events associated with integrin activation via induction of PKCβIII -mediated L-plastin phosphorylation.


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EOSINOPHILS, EOSINOPHIL DEGRANULATION AND RESPIRATORY VIRUS INFECTION IN VIVO

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Background: Studies performed in vitro suggest that human eosinophils, via their degranulation products, can reduce the infectivity of the human respiratory syncytial virus pathogen (RSV) for target epithelial cells in culture [1, 2]. Eosinophil-dependent antiviral activity against RSV and Sendai virus has been documented in IL-5 transgenic and allergen-challenged mice [3, 4]. We have not observed similar antiviral activity in vivo in infection studies with pneumonia virus of mice (PVM), a pneumovirus pathogen that undergoes robust replication in mouse lung tissue. In order to examine this further, we evaluated PVM infection in highly hypereosinophilic eotaxin-2 (hE2) / interleukin-5 (IL-5) double transgenic (hE2IL5tg) mice [5]. In these mice, which express interleukin-5 systemically and eotaxin-2 via the pulmonary specific Clara cell CC10 promoter, eosinophils are recruited to the lung and airways and are actively degranulating, similar to what is observed in human asthma.

Objectives: Using the hE2IL5tg mice, we will examine the impact of pulmonary eosinophils and eosinophil degranulation on the pathogenesis of PVM infection in vivo.

Methods: hE2IL5tg, hE2tg, IL5tg, and wild type mice were inoculated intranasally with 1.7 TCID50 units of PVM in a 50 μL inoculum. Cell differential was assessed in bronchoalveolar lavage (BAL) fluid recovered five days after PVM inoculation; ribonuclease (RNase) activity characteristic of eosinophil granule ribonucleases (mEars) was determined in cell-free BAL fluid. Virus titer was determined by quantitative RT-PCR targeting the PVM small hydrophobic (SH) gene. EPO release was determined as described by Adamko and colleagues [6].

Results: The eosinophil is the predominant cell detected in BAL fluid of PVM-infected hE2IL5tg mice. In contrast to other mice, these eosinophils have undergone substantial degranulation by visual inspection, although eosinophils from the BAL fluid retained 90% of their original EPO content. Virus recovery from lung tissue of hE2IL5tg mice is significantly reduced (nearly three-logs) when compared to those from infected wt, hE2tg, IL5tg mice; ribonuclease activity in BAL fluid correlates directly with the eosinophil count in these individual mouse strains. Experiments underway will address the direct relationship between eosinophil degranulation, ribonuclease activity, and virus recovery from lung tissue of PVM-infected mice.

Conclusions: Virus recovery is diminished in lung tissue in hE2IL5tg mice, a model of pulmonary eosinophilia that focuses on active degranulation. With this model, we continue our exploration of the antiviral features of eosinophil secretory ribonucleases and other degranulation products.

References:

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POSTER A-16
DIFFERENTIAL EFFECT OF EOTAXINS ON HUMAN EOSINOPHIL MIGRATION:
EOTAXIN-3 ACTIVATES ANOTHER RECEPTOR BESIDES CCR3

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Background. Eotaxin-1, -2 and -3 are potent chemokines mainly attracting eosinophils which are involved in asthmatic inflammation. They activate a common receptor, namely CCR3. In this study we compared the effects of eotaxins on human eosinophil migration.

Methods. Eosinophils were isolated from the blood of healthy and asthmatic volunteers and migration assays through cell culture inserts coated with a reconstituted basement membrane (Matrigel™) were performed.

Results. All eotaxins induced a similar migration pattern of asthmatic eosinophils up to 6 hours. Interestingly, only eotaxin-3-induced a second migration burst between 12 and 18 hours. CCR3 blockade almost completely inhibited the migration of asthmatic eosinophils induced by eotaxin-1 and eotaxin-2 while partially blocking that induced by eotaxin-3. Kinetic analyses showed that CCR3 blockade inhibited the early (0-6 hours) but not the later (9-18 hours) phase of eotaxin-3-induced migration. Moreover, asthmatic eosinophils had an enhanced response to eotaxin-3 compared to those of healthy volunteers. Finally, the migration rates of eosinophils from healthy volunteers were similar for all eotaxins and completely inhibited by CCR3 blockade.

Conclusion. These results show that eotaxins induce a similar migration pattern in the early phase of asthmatic eosinophil migration (0-6 hours) and that an additional migration of asthmatic eosinophils is observed only with eotaxin-3. This suggests that eosinophils from asthmatics but not those of healthy volunteers express an additional receptor that is activated by eotaxin-3 but not by eotaxin-1 or -2. This additional receptor, which might be involved in asthma, would explain the greater efficacy of eotaxin-3 to induce the migration of eosinophils from asthmatics compared to healthy subjects.
**Background:** Thymic stromal lymphopoietin (TSLP) is recognized as an important component of allergic inflammation. Both T cells and mast cells are activated by TSLP, suggesting multiple pathways through which TSLP can influence allergic inflammation. TSLP signals through IL-7 receptor-alpha chain (IL-7Rα) complexed with TSLP receptor (TSLPR). Eosinophils have previously been shown to express IL-7Rα and, upon TSLP stimulation, to secrete cytokines and have enhanced viability.

**Objectives:** As TSLP and eosinophils are prominent components of allergic inflammation, we sought to determine whether TSLP could induce additional eosinophil functions by examining degranulation, STAT5 signaling, superoxide production and chemotaxis.

**Methods:** Eosinophils were isolated from heparinized human peripheral blood via magnetic bead negative selection. The resulting cells were > 99% pure and > 97% viable. TSLPR and IL-7Ra mRNA expression were examined by real-time quantitative PCR of eosinophils treated with various combinations of IL-3, IL-5, GM-CSF, and TNFα for 24 hrs. Time courses and concentration curves were also conducted for the combination of IL-3 and TNFα. TSLPR expression on eosinophils was analyzed by flow cytometry and western blots. TSLP stimulated eosinophils were evaluated for release of eosinophil derived neurotoxin (EDN) by ELISA, phosphorylation of STAT5 by intracellular flow cytometry and survival/viability by trypan blue exclusion, superoxide production by cytochrome C reduction, and chemotaxis by transwell migration. Eosinophil degranulation, superoxide release, chemotaxis, and TSLPR surface expression were evaluated with and without pre-activation with TNFαs and IL-3. A functional blocking antibody for TSLPR was used to confirm the specificity of TSLP mediated signaling on eosinophil degranulation.

**Results:** Eosinophil expression of cell surface TSLPR and TSLPR mRNA was upregulated by stimulation with TNFα and IL-5 family cytokines, with the combination of TNFαs and IL-3 resulting in the greatest response. IL-7Ra mRNA was detectable and not upregulated by TNFαs and IL-5 family cytokines. TSLPR upregulation was increased at 4 hrs by stimulation with TNFαs and IL-3 and remained elevated through 48 hrs. TSLP stimulation resulted in release of EDN, phosphorylation of STAT5 as well as promotion of eosinophil viability and survival, but not superoxide production or chemotaxis. TSLP induced dose dependent survival and viability were significant at 48 and 72 hrs. Furthermore, TSLP-stimulated eosinophil degranulation was inhibited by a functional blocking antibody to TSLPR. Pre-activation of eosinophils with TNFαs and IL-3 resulted in eosinophil degranulation at lower concentrations of TSLP.

**Conclusions:** This study demonstrates that eosinophils respond directly and selectively to TSLP, indicating that the eosinophil has the capacity to participate in TSLP-driven allergic responses.

**Grant Support:** This work was supported by NIH HL088584, NIH EY012526, and a UW Hilldale Undergraduate Research Fellowship.
POSTER A-18

CCR3-MEDIATED MOUSE EOSINOPHIL DEGRANULATION; REGULATION BY INTEGRIN, MICROTUBULES AND CYTOSKELETAL MACHINERY

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Background: Eosinophils are end-stage effector cells that following stimulation secrete cytotoxic proteins and inflammatory mediators from their cytoplasmic granules as part of their roles in host defense response, immunomodulation and tissue remodeling. Mechanisms of eosinophil degranulation have been studied principally in human eosinophils, and little in mouse eosinophils, the major in vivo model for eosinophil-mediated disease.

Objectives: Our study aims to reveal the mechanisms of eosinophil degranulation/secretory responses of murine eosinophils following physiological stimulation by cytokines and chemokines and the involvement of cytoskeleton and microtubule machineries in this process.

Methods: Mouse eosinophils were purified from IL-5 transgenic mouse spleens. Degranulation responses of mouse eosinophils stimulated with eotaxin 1 (CCL11) and 2 (CCL24) were assayed mainly by measuring the secretion of eosinophil-associated RNases (EARs) and validated by assays of secreted eosinophil-peroxidase activity and major-basic protein. In order to reveal the role of various signaling and adhesion molecules as well as key factors of cytoskeletal machinery and microtubule polymerization in eosinophil degranulation, we used specific inhibitors and blocking antibodies.

Results: Following stimulation with CCL11 and CCL24, mouse eosinophils undergo piecemeal degranulation mediated by Gαi and phosphoinositide 3-kinase. Adhesion molecules were found to be crucial for CCR3-mediated degranulation, since blocking beta 1 and 2 integrins abolished EAR secretion. Dynamic microtubule polymerization was also found to be essential for degranulation. However, inhibition of actin polymerization as well as inhibition of regulatory components of the cytoskeletal machinery, such as Rac1 and Rho kinases (ROCK-I and –II), increased EARs secretion in mouse eosinophils, suggesting a negative regulation of these effectors. Interestingly, cell-free granules purified from mouse eosinophils were found to express functional CCR3 and secreted mEARs in response to CCL11, suggesting an additional mechanism of degranulation in a cell-free context.

Conclusions: Collectively, these data demonstrate that mouse eosinophils undergo degranulation in response to physiological stimulation with CCL11 and CCL24, in an integrin- and microtubule-dependent manner. Cytoskeletal machinery negatively regulates degranulation, suggesting that the cytoskeleton prevents spontaneous degranulation.

Grant Support: This work is funded by grants from NIH (R01/R37 AI020241, R01 AI05164).
Background: HMGB1 is an evolutionarily conserved multifunctional protein initially recognized as a chromosomal structural protein. Subsequent findings established that HMGB1 also translocates out of the nucleus after modification, either passively during cellular necrosis, or actively in response to cytokine stimulation, resulting in its extracellular secretion as a proinflammatory mediator. Our previous proteomic studies of human blood eosinophils identified HMGB1 in these granulocytes [1]. This observation prompted us to investigate the activity of HMGB1 further. Previous reports have shown that the proinflammatory action of HMGB1 is mediated through toll-like receptors (TLR) 2 and 4 as well as the receptor for advanced glycation end products (RAGE).

Objectives: In the long term we plan to study the involvement of HMGB1 in airway disease. The achievement of this goal requires the characterization of HMGB1 activity relating to eosinophil function. Thus, the translocation and secretion of HMGB1 from eosinophils was evaluated by stimulation with GM-CSF, TNF-α, and IFN-γ. We also investigated the autocrine role of HMGB1 in eosinophil activation and cell survival. Additional investigations included characterizing HMGB1 receptors on eosinophils.

Methods: Peripheral blood eosinophils were isolated from non-allergic donors using CD16/CD14 negative selection (>98% pure, CD69 negative cells). Recombinant endotoxin-tested HMGB1 was produced in-house and characterized by 1D and 2D gel electrophoresis, reversed-phase HPLC, and mass spectrometry. HMGB1 autocrine activity was assessed by measuring eosinophil survival after 24 h using Annexin V/PE staining. The HMGB1 inhibitor, glycyrrhizin, and anti-RAGE were also employed. Subcellular translocation and active secretion of HMGB1 from eosinophils stimulated with GM-CSF, TNFα, and IFN-γ was evaluated by immunoblotting after 1D and 2D gel electrophoresis of lysates and of subcellular fractions. Flow cytometry was used to simultaneously evaluate the expression of eosinophil HMGB1 receptors and the activation marker CD69 in response to GM-CSF stimulation.

Results: Purified eosinophils were virtually devoid of CD69, CD14, TLR-2 and TLR-4. Upon cytokine stimulation of eosinophils for 2 h, significant subcellular HMGB1 translocation was detected by immunoblotting and HMGB1 secretion could be enhanced by prolonged stimulation (GM-CSF, 24 h). Activation of eosinophils by HMGB1 was demonstrated by prolonged survival and CD69 induction. Importantly, prolongation of survival was significantly inhibited by glycyrrhizin and partially by anti-RAGE. TLR-2, TLR-4, and CD69, expression on eosinophils were inducible by stimulation with GM-CSF (10 ng/ml), showing prolonged induction over 2–18 h.

Conclusions: Our previous proteomic studies of eosinophils [1], identifying the presence of HMGB1 in eosinophils, prompted investigations into HMGB1 activity and raised questions about HMGB1’s potential involvement in inflammation associated with airway disease, especially allergy and asthma. This interest inspired us to conduct studies in which we determined that eosinophils are capable of actively secreting HMGB1 and can also respond to rHMGB1 in an autocrine fashion.


Grant Support: This work is funded by NIH/NHLBI N01-HV-00245 (A. K.), NIH/NCRR KL2RR029875 (K. P.) and a pre-doctoral McLaughlin Foundation fellowship (C. S.).
POSTER A-20

EOSINOPHIL EXTRACELLULAR DNA TRAP CELL DEATH INDUCES THE INTACT GRANULE RELEASE THROUGH DISTINCT MECHANISMS

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Background: Depositions of Clusters of free, extracellular eosinophil granules (Cfegs) are recognized in the airways or tissues in association with diverse disorders, including allergic asthma and rhinitis, dermatitis, helminth infections, eosinophilic esophagitis and urticaria. We recently established that cell-free eosinophil granules express ligand-binding cytokine, chemokine and eicosanoid receptors that activate intragranular signaling to stimulate granules to directly secrete selected granule-derived proteins1, 2. However, the mechanism of cytolysis of eosinophils that produces intact membrane-bound granules is not understood.

Objectives: The aim of this study is to establish an in vitro model of Cfegs formation using the calcium ionophore A23187, a known eosinophil lytic stimulus3.

Methods: Human peripheral blood eosinophils were purified by negative selection. Viability and morphology of Cfegs were assessed by CYTO64, CYTOX, Hoechst, acridine orange, Lysotracker, Celltracker, and Annexin V stainings. Counting and characteristics of Cfegs were studied by flow cytometry and fluorescence microscopy. Release of granules was also studied by time-lapse phase contrast microscopy.

Results: Eosinophils stimulated with A23187 for 1 h showed rapid cell death with release of extracellular DNA traps from nucleus, which was not observed with heat-shocked necrotic and Fas-activated apoptotic eosinophils. A23187-induced cell death was inhibited by the calcium chelator, BAPTA-AM; the NADPH oxidase inhibitor, diphenyliodonium (DPI); and fetal bovine serum (FBS). A23187 stimulation, like heat-shock, induced eosinophils to release Annexin V negative structures. In contrast, Fas-activated cells produced Annexin V positive structures (apoptotic bodies). A23187-induced structures were revealed as Cfegs (or intact granules), evidenced by a positive staining with acidic dyes and MBP. Number of Cfegs in the culture medium was increased by A23187 stimulation and the production was inhibited by BAPTA-AM, DPI, and FBS. Interestingly, some packed granules were found in A23187-stimulated culture supernatants, with Celltracker positive cytosol and expression of surface MHC class I, a plasma membrane marker. Time-lapse imaging revealed that some “membrane-enveloped” granules were produced by plasma membrane protrusion and separation, and plasma membrane-free “bare” granules were produced by plasma membrane rupture.

Conclusions: Our results indicate that 1) calcium overload induces ROS dependent typical extracellular DNA trap cell death, and 2) this cell death caused intact granule release as either “bare granules” or as the contents of plasma membrane-bound structures. Membrane vesicle production might be a novel secretion/degranulation process associated with active cell death.

References:

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9-CIS RETINOIC ACID UP-REGULATES FUNCTIONAL ICAM-1 ON HUMAN EOSINOPHILS

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Background: Eosinophils are resident immune cells in intestine. Retinoic acids (RAs), which are active metabolites of vitamin A, play critical roles in mucosal immune responses, particularly in the intestine. We have reported that RAs dramatically prolong human eosinophil survival, and screened ICAM-1 (CD54) up-regulation in RA-stimulated eosinophils using gene microarray. ICAM-1 is known to play important roles as a co-stimulating molecule in antigen presentation and also in maintenance of eosinophil survival.

Objectives: The aim of present study is to clarify the effect of retinoic acid on expression of functional ICAM-1 on human eosinophils.

Methods: Expression of ICAM-1 was studied using real-time PCR and flow cytometry. Pharmacological agonists and inhibitors were used to study the involvement of specific nuclear receptors for retinoid acids. ICAM-1 was activated by crosslinking, and phosphorylation of MAP kinases and Akt signaling were studied using multiplex bead assay.

Results: We first confirmed that eosinophils cultured with all-trans RA (ATRA) and 9-cis RA significantly induced expression of ICAM-1 at the mRNA level. Consistent with this, although purified eosinophils expressed low levels of surface ICAM-1, 9-cis RA and ATRA up-regulated ICAM-1 in a concentration dependent manner. TTNPB, a retinoic acid receptor (RAR) agonist, mimicked the effect; however, HX630, a retinoid X receptor (RXR) agonist, did not. Further, RAR antagonist HX531 completely blocked RA-induced ICAM-1 expression, suggesting that RAR is responsible for the effect. Up-regulated ICAM-1 appears to be functional because ICAM-1 crosslinking resulted in enhanced phosphorylation of Akt, ERK, and p38.

Conclusions: RAs might play a role in mucosal immunity through functionally up-regulating eosinophils.

References:

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POSTER A-22

EOSINOPHIL ACTIVATION AND SECRETORY “DEGRANULATION” ELICITED BY SIGNALING LYMPHOCYTE ACTIVATION MOLECULE (SLAM) FAMILY RECEPTOR 2B4

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Background: Eosinophils play critical roles in allergic inflammation, parasitic infections and other immunomodulatory functions by releasing dozens of pro-inflammatory and immunoregulatory cytokines. However, mechanisms of eosinophil activation and cytokine secretion are still poorly understood. Signaling lymphocyte activation molecule (SLAM) family receptor 2B4, mainly expressed by NK cells and T cells as an activation molecule, has been more expansively recognized to be expressed by monocytes, basophils and human eosinophils.

Objectives: To explore the expression and function of the SLAM receptor, 2B4, on mouse eosinophils.

Methods: Expression of 2B4 on mouse eosinophils purified from the spleens of IL-5 transgenic mice was assessed by flow cytometry and Western blotting. To investigate 2B4 function on eosinophils, we incubated eosinophils with rat anti-mouse 2B4 (C9.1) or isotype-matched control Ab following a goat anti-rat IgG for 16h. Supernatants were collected and cytokine profiles were measured by Bio-plex assays.

Results: 2B4 protein was constitutively expressed and displayed on the plasma membranes of mouse eosinophils and could be up-regulated by recombinant mouse GM-CSF, as assessed by both flow cytometry and Western blotting. Cross-linking of eosinophil, plasma membrane expressed surface 2B4 activated eosinophils to release significant amounts of eosinophil-derived cytokines, including IL-2, IL-4, IL-5, IL-10, IL-12 and INF-g.

Conclusions: Mouse eosinophils express functional 2B4 cell-surface receptors that can activate eosinophils and trigger their secretion of their diverse content of pre-formed cytokines. Mouse eosinophils, like human eosinophils, are sources of pre-formed secretable cytokines; and engagements of the SLAM family receptor protein, 2B4, on mouse eosinophils can regulate the secretion of eosinophil-derived cytokines.

Grant support: NIH-R37AI020241 and NIH-R01AI051645 (PFW).
POSTER A-23
COD FISH IS A CHEMOATTRACTANT FOR HUMAN EOSINOPHILS
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Background: Cod fish is an important food allergen in Scandinavia. Eosinophilic granulocytes recognize and are directly activated by aeroallergens [1, 2].

Objectives: To determine if eosinophils recognize and become activated by food allergens in the absence of other mediators and cells.

Methods: Freshly purified eosinophils from non-allergic donors are exposed to cod fish extract, and their ability to produce free oxygen radicals (chemoluminescence), degranulate EPO (in-house enzyme assay), EDN (ELISA) and ECP (Uni-CAP FEIA), release Ca²⁺ intracellularly (flow cytometry), and migrate in vitro (Neuroprobe 3 mm chemotaxis plates) is assessed. Desensitization experiments are performed by pre-incubation of eosinophils with fMLF, C5a, PGD2 and CCL11, followed by incubation of the cells with cod fish.

Results: Eosinophilic granulocytes readily migrate toward cod fish but do not degranulate nor do they produce free oxygen radicals in response to cod fish. Exposure to cod fish causes rapid release of intracellular calcium. The cod fish response evoked in human eosinophils was very strong, able to desensitize the responsiveness of eosinophils to fMLF, PGD2 and CCL11, but not to C5a.

Conclusions: We have shown that cod fish is a powerful chemoattractant for human eosinophils. Our goal is to identify the bioactive substances responsible for the chemoattractant activity of cod fish.


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NOVEL ELISA FOR EOSINOPHIL PEROXIDASE IS A SPECIFIC AND SENSITIVE METHOD OF DETECTION OF EOSINOPHIL DEGRANULATION IN HUMAN BIOLOGICAL FLUID SAMPLES.

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Background: Normally eosinophils comprise 1-3% of circulating leukocytes with absolute eosinophil counts generally below 600 cells/μl of blood. Elevations in the numbers of these leukocytes (i.e., eosinophilia) is associated with a variety of inflammatory and disease states, including parasitic and fungal infections, allergic diseases such as asthma, rhinitis and sensitivities to specific foods, cancer, and transplant rejection. The accurate evaluations of the presence of tissue infiltrating eosinophils as well as their activation state are important for the monitoring of disease progression and effectiveness of the treatment(s) employed. The assessment of the presence of eosinophil peroxidase (EPX), a specific marker of eosinophil activation/degranulation, may be an invaluable tool for such evaluations.

Objective: The aim of this study was to develop a sensitive and specific ELISA for detection of human EPX that would be applicable for assaying biological fluids and tissue extracts.

Methods: We have developed highly specific antibodies that recognize EPX that with human biopsies were subsequently efficiently used for immunohistochemistry [1]. These antibodies were subsequently used to develop a sandwich ELISA for the detection of EPX in biological fluid samples. Using full factorial design of experiments we optimized our previously developed mouse EPX ELISA protocol for detection of purified human EPX (St. Louis, Missouri, USA) as well as EPX in extracts from highly purified human eosinophils.

Results: Our human EPX ELISA assay was as sensitive as the earlier mouse EPX ELISA when used with extracts of purified eosinophils from respective species. EPX ELISAs per mole sensitivity was comparable to the one of commercially available ECP and EDN ELISAs (MBL, Woburn, MA, USA) with the additional advantage of being eosinophil specific. The assay is able to readily detect degranulation of human eosinophils stimulated ex-vivo as well as detect the presence of EPX in sputum of asthma patients, in serum of patients with normal end elevated eosinophil count, and in stomach fluid from a patient with stomach eosinophilia.

Conclusions: We have developed a novel diagnostic sandwich ELISA that specifically detects and quantifies eosinophil degranulation in human samples.


Grant Support: This work is funded by Mayo Foundation for Medical Education and Research and NIH: NCRR K26 RR0109709 and NCI R01CA112442.
NOVEL ELISA FOR EOSINOPHIL PEROXIDASE IS A HIGHLY SPECIFIC SENSITIVE TEST FOR EOSINOPHIL DEGRANULATION IN THE MOUSE.

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**Background:** Mouse models of eosinophilic disorders are powerful tools to study the underlined biological mechanisms and have led to discoveries of new therapeutic modalities. The presence of eosinophil peroxidase (EPX) in the affected tissues is a well established specific marker of eosinophil degranulation [1]. Therefore, specific identification of this molecule in biological fluids and tissue extracts may prove invaluable for study of eosinophil associated diseases in the mouse.

**Objective:** Our goal was to develop a specific and sensitive assay for the presence of eosinophil peroxidase in the mouse fluid samples.

**Methods:** We chose a sandwich ELISA format to detect EPX because (1) it has the advantage of doubled specificity associated with analyte recognition via employing two specific antibodies, and (2) the ability to detect EPX in biological fluids and tissue extracts irrespective of EPX activity. The assay was developed using alkaline phosphatase ELISA reagents from KPL (Gaithersburg, MD, USA), and two specific anti-eosinophil peroxidase antibodies developed in our laboratory. We used full factorial design of experiments and JMP (SAS Institute, Cary, NC) statistical software package for optimization of the assay protocol.

**Results:** Our EPX ELISA assay is ~ 10 times more sensitive than the traditional OPD-based EPX activity assay. Specificity was confirmed in experiments using EPX deficient and eosinophil deficient mice. The assay could readily detect eosinophil EPX release from purified mouse eosinophils stimulated ex-vivo with platelet activating factor (PAF) and ionomycin. Unlike traditional OPD based EPX activity assay, our ELISA assay could easily detect eosinophil degranulation in bronchoalveolar lavage of mice after acute ovalbumin sensitization/challenge protocol. The assay was also successfully used to detect EPX in serum of transgenic hypereosinophilic mice.

**Conclusions:** We have developed a highly sensitive and specific EPX ELISA that can be used for ex-vivo and in vivo studies of eosinophil associated pathologies in mouse.


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POSTER B-1

PTP1B DEFICIENCY ELEVATES EOSINOPHIL PROGENITORS AND ACCELERATES LEUKOCYTE RECRUITMENT DURING ALLERGIC INFLAMMATION

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Background: It is reported that protein tyrosine phosphatase 1B (PTP1B) limits cytokine responses. However, PTP1B’s function in eosinophil recruitment during allergic inflammation is not known.

Objective: We hypothesize that PTP1B deficiency elevates eosinophilic recruitment in the OVA model of allergic inflammation.

Methods: PTP1B-/- and wild type mice (BALB/c background) were from Dr. Michel Tremblay (McGill University, Canada). Ovalbumin-induced allergic inflammation models. Mice were sensitized by i.p. injection (100 ml) of OVA 10mg/alum or saline/alum on days 0 and 7 and then challenged on days 15, 17, and 0 with intranasal OVA (0mg in 0 ml saline) or 0 ml saline alone. Inflammation was examined either 4 hours after the first OVA challenge (day 16) or 24 hours after the third OVA challenge (day 21). On day 16 or 21, the lungs were lavaged with 0.5 ml ice-cold PBS to obtain bronchoalveolar lavage (BAL) cells; one lobe was harvested for cytokine/chemokine analysis; the rest of the lung was frozen in OCT and used for H&E staining of tissue sections. Cell cytospins were DiffQuick stained and cells were counted according to standard morphological criteria. OVA-specific IgE was determined by ELISA. Blood, BAL and spleen leukocytes were isolated, immunolabeled and examined by flow cytometry.

Intravital microscopy: Mice were sensitized by intraperitoneal (i.p.) injection of OVA 10mg/alum or saline/alum on days 0 and 7. On day 17 mice were anesthetized and challenged by intradermal injection of OVA in saline in one ear or saline alone in the other ear. The mice were injected retroorbitally with rhodamine 6G to label circulating leukocytes in the blood. The ears were then examined by intravital microscopy for leukocyte-endothelial cell interactions (numbers of leukocytes rolling, leukocyte rolling velocity, leukocyte adhesion).

In vitro cell adhesion and migration assays with laminar flow. A parallel plate flow chamber was used to examine leukocyte migration across endothelium under conditions of laminar flow of 2 dynes/cm2. Leukocyte association was examined at 2 min and leukocyte transendothelial migration was examined at 15 min.

Results: There was an OVA-induced increase in numbers of SiglecFhighCD34+IL-5Rα eosinophil progenitors in the peripheral blood and spleens of PTP1B-/- mice compared to WT mice. In lungs, OVA-challenged PTP1B-/- mice had elevated numbers of eosinophils and eosinophil progenitors at 6 hours after one OVA-challenge and at 24 hrs after a third OVA challenge as compared to OVA-challenged wild type mice. Saline-treated PTP1B-/- mice had no change in basal lung inflammation compared to WT mice. Intravital microscopy revealed that, in OVA-challenged PTP1B-/- mice, blood leukocytes bound to endothelium within 5-30 minutes, whereas, in wild type mice, blood leukocytes bound to endothelium at the expected 6-18 hrs after OVA challenge. Consistent with early recruitment of leukocytes, lung eotaxin and Th2 cytokine levels were elevated in the PTP1B-/- mice at 6hrs after one challenge. In vitro, spleen leukocytes from PTP1B-/- mice exhibited increased migration across wild type endothelial cells.

Conclusions: In summary, PTP1B functions as a critical negative regulator to limit eosinophilic inflammation.

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**POSTER B-2**

**IL-4 AND IL-33 INDUCE EOSINOPHIL PRODUCTION OF RELM-α BY A TYPE I IL-4R-DEPENDENT MECHANISM**

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**Background:** Eosinophils are known to be major effector cells in type 2 inflammatory diseases such as asthma, allergies and parasitic infections. Microarray mRNA expression analysis revealed that Relm-α was highly expressed in murine eosinophils from asthmatic lungs compared to eosinophils from non-allergen-treated control mice, suggesting that Relm-α may be an important gene product of activated eosinophils. Relm-α belongs to a newly defined family of resistin-like molecules including Relm-β and Relm-γ. Although it has been reported that Relm-α is associated with pulmonary inflammation, its specific role and regulation remain unclear [1]. Interleukin (IL)-33, a newly identified cytokine, has been recently shown to be involved in airway inflammation by inducing Th2 cytokines, including IL-4, IL-5 and IL-13, which collectively have a crucial role in the pathogenesis of allergic airway inflammation [2].

**Objective:** We hypothesized that the inflammatory cytokines IL-4, IL-5, IL-13 and IL-33 may have an effect on Relm-α production by eosinophils.

**Methods:** Murine eosinophils were generated from bone marrow progenitors derived by culture of low density bone marrow cells in the presence of 100 ng/ml of both stem cell factor (SCF) and fetal liver tyrosine kinase (FLT)-3 ligand for 4 days. Subsequently, cells were incubated with 10 ng/ml of IL-5 for 10 days. After generation at day 14, eosinophils (4x10⁶ cells) were incubated with or without (control non-activated) murine recombinant cytokines for 18h. Relm-α expression at the mRNA or protein level was measured by qPCR or ELISA, respectively.

**Results:** IL-4 and IL-33 but neither IL-5 nor IL-13 induced Relm-α production by eosinophils. To determine whether type I or type II IL-4R was involved in the activation, we generated eosinophils from IL-4Rα-/- and IL-13Rα1 -/- mice. Compared to wild-type (WT) and IL-13Rα1 -/- eosinophil cultures, the production of Relm-α was abolished in IL-4Rα-/- eosinophils. Surprisingly, we discovered that IL-33 lost its ability to induce Relm-α in IL-4Rα-/- eosinophils despite the fact that IL-33 induced the production of IL-4 in IL-4Rα-/- as well as WT and IL-13Rα1 -/- mice.

**Conclusion:** Taken together, our results demonstrate that IL-4 and IL-33 induce eosinophil production of Relm-α by a type I IL-4R-dependent mechanism. Further, we propose that IL-33 stimulates IL-4 production, which subsequently promotes type I IL-4R-dependent Relm-α production. As such, we have identified a novel activation pathway in murine eosinophils.

**References:**

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POSTER B-3

ASYMPTOMATIC HYPEREOSINOPHILIC SYNDROME: A BENIGN DISORDER?

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Background: Hypereosinophilic syndrome (HES) is characterized by peripheral eosinophilia >1,500/mm³ and a variety of clinical manifestations. Although most patients with HES present with clinical signs and symptoms attributable to eosinophilic tissue infiltration, some untreated patients remain asymptomatic or have signs and symptoms, such as allergic rhinitis, for which the relationship to peripheral eosinophilia is unclear. Whether these patients have early HES or a benign form of hypereosinophilia is unknown.

Objectives: To determine the prevalence, clinical and immunologic characteristics and long-term prognosis of patients presenting with asymptomatic eosinophilia >1,500/mm³ of ≥3 years duration.

Methods: Subjects referred to the NIH for evaluation of unexplained eosinophilia were followed prospectively on a yearly basis. Clinical evaluation included history, physical examination, routine laboratory testing and assessment of end organ involvement. Whole blood flow was performed for lymphocyte phenotyping and assessment of eosinophil activation. Serum was collected for measurement of eosinophil granule proteins, TARC and soluble IL5 receptor levels by ELISA. Peripheral blood mononuclear cells were cultured overnight in the presence and absence of PMA/ionomycin. Cytokine production was assessed by intracellular flow cytometry and analysis of PBMC supernatants using suspension array technology in multiplex. Results are expressed as net levels (stimulated-unstimulated).

Results: Of 231 subjects with eosinophilia >1500/mm³ evaluated since 1994, 6 were found to have asymptomatic, unexplained eosinophilia of ≥3 years duration (range 3-29 years). Five of the 6 subjects were male, and the median age at presentation was 31 (range 16-53). Geometric mean peak eosinophil count was 4201/mm³ (range 2061-7170/mm³). IgE levels were moderately elevated (<1000 ng/ml) in 4 subjects and normal in 2 subjects. Although the eosinophil count has decreased over time in 5/6 subjects, it has remained above 1,500/mm³ in 3 subjects, above 1,000 in 2 additional subjects and 690/mm³ in the final subject. Evidence of eosinophil activation, as assessed by increased surface expression of CD25, CD69, and/or HLA-DR, was seen consistently in only 2/6 subjects. Two subjects had a clonal T cell population detected by PCR, one of whom had an aberrant CD3-CD4+ T cell population confirmed by flow cytometry. Serum levels of TARC, a marker of lymphocyte activation, were elevated (>1000 pg/ml) at all time points in 3 subjects, including both of those with clonal T cell populations. Among the cytokines measured, only IL-5 and IL-9 levels were significantly increased in stimulated PBMC from subjects with benign eosinophilia as compared to those from normal controls.

Conclusions: A small number of patients with persistent peripheral eosinophilia ≥1,500/mm³ appear to have clinically benign disease that is accompanied by a relative lack of eosinophil activation as assessed by flow cytometry. Preliminary data suggests that eosinophilia in these subjects may be lymphocyte-driven.

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POSTER B-4

EOSINOPHILS ARE ESSENTIAL FOR THE LONG-TERM SURVIVAL OF PLASMA CELLS IN THE BONE MARROW

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Background: It is thought that in the bone marrow (BM) a network of reticular stromal cells provides a survival niche which supports the long-term maintenance of plasma cells. The mutual interaction between stromal cells and plasma cells is a pre-requisite for the longevity of plasma cells. In addition, plasma cells require cytokines, such as APRIL and IL-6 secreted by eosinophils to survive for weeks or even life time in the BM.

Objectives: We asked the question whether depletion of eosinophils effects to the survival of long-lived plasma cells in the BM.

Methods: APRIL/and IL-6 high producing cells are analyzed in the BM from wild type or eosinophil-deficient mice by immunohistochemical staining. BALB/c mice are immunized with the T cell-dependent antigen 2-phenyl-oxazolone (phOx) delivered i.p. in alum or CFA (10) and secondary boost given with soluble phOx (20). The number of eosinophils in the BM is analyzed by using flow cytometry. Eosinophils isolated from the BM of naive, 10 and 20 immunized BALB/c mice are compared the levels of IL-4, APRIL and IL-6 expression using bioplex assay or RT-PCR. To do kinetics of eosinophil depletion, BALB/c mice are injected with anti-Siglec-F or rat isotype control for three times per week. The number and frequency of eosinophils are determined at different time points. In addition, BALB/c mice are immunized 10 and 20, 60 days after boosting antigen allows long-lived plasma cells established in the BM. The mice are treated with anti-Siglec-F or isotype control, the titer of antigen-specific antibodies, the number and the percentage of Annexin-V+ plasma cells are determined1.

Results: Our analysis of the BM showed that eosinophils are the main source of the plasma cell survival factors APRIL and IL-6. We find that upon immunization with a T cell-dependent antigen, the number of eosinophils is increased by adjuvant dependent and independent ways. In addition, eosinophils are activated and secretion of APRIL and IL-6 is enhanced. When mice are treated with anti-Siglec-F antibody the number of eosinophils is completely reduced in the BM, blood and spleen. The effect of depletion continues for 2 weeks, afterwards eosinophils are recovered. In the BM, eosinophils are required for the accumulation of plasma cells1. Interestingly, depletion of eosinophils reduced the number of long-lived plasma cells in the BM by promoting apoptosis. Eosinophils are essential for the long term survival of plasma cells.

Conclusions: We have shown that the long-term maintenance of plasma cells in the BM requires eosinophils. Eosinophil-directed therapy might provide a novel therapeutic approach to treat autoimmune and other diseases where plasma cells contribute to the pathogenesis.


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THE EXPRESSION OF THE ABUNDANT SECONDARY GRANULE PROTEINS MBP-1 AND EPO IS REQUIRED FOR THE MATURATION OF EOSINOPHIL-LINEAGE COMMITTED PROGENITOR CELLS IN THE HEMATOPOIETIC COMPARTMENTS OF MICE

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Background: Eosinophil effector functions have been hypothesized to be mediated in part by the release of proteins stored in the secondary granules of mature cells of this leukocyte lineage. Major basic protein-1 (MBP-1) and eosinophil peroxidase (EPO) comprise the majority of protein by mass in these granules. No role has been speculated for these proteins in eosinophilopoiesis and/or the accumulation of mature cells in circulation.

Objectives: We demonstrate that, unlike the single deficiency of MBP-1 or EPO, the absence of both granule proteins resulted in the loss of peripheral blood eosinophils.

Methods: Eosinophilopoiesis and eosinophil accumulation in the periphery were assessed in single and double knockout (MBP-1-/- and/or EPO-/-) mice. Bone marrow and peripheral blood leukocytes were assessed by IHC, FACS and in vitro cell culture assays. Mice were subjected to an acute OVA protocol assessing induced pulmonary pathologies in each of the strains of granule protein knockout mice.

Results: IHC assessments of bone marrow and spleen demonstrate that eosinophil lineage commitment occurs in these mice. However, these assessments and FACS studies demonstrated a blockade in the maturation of eosinophil-lineage committed cells. This blockade is not rescued by ex vivo culture or by bone marrow engraftment into wild type recipient mice. Similar to other eosinophil-less mouse models (ie., PHIL and ΔdblGATA), MBP-1-/-/EPO-/- mice also fail to develop pulmonary inflammation in an OVA protocol.

Conclusions: These results demonstrate that the combined loss of MBP-1 and EPO disrupts the generation of mature eosinophils in the mouse.

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POSTER B-6
MEASURING EOSINOPHIL KINETICS IN HUMANS

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Background: Eosinophils are major effector cells in allergic inflammation and represent an important therapeutic target in asthma. While much is understood about the mechanisms controlling the generation and activation of eosinophils, little is known about their intravascular kinetics and physiological fate.

Objectives: The purpose of this study was to use minimally manipulated 111-Indium-labelled eosinophils to determine the intravascular half-life of eosinophils and the extent of eosinophil intravascular margination.

Methods: Leukocytes were isolated from healthy volunteers, labelled with 111-Indium-tropolonate and re-injected. Blood was sampled at 0.75, 2, 4, 6, 9, 12, 24, 48 and 72h post-injection. From each sample, neutrophils and eosinophils were isolated in parallel using Robosep® negative selection, and cell-associated radioactivity quantified to determine intravascular recovery and lifespan.

Results: Neutrophils gave a 45-minute recovery of 57 ± SEM 10% and left the circulation monoexponentially with a mean lifespan of 10.3 ± SEM 0.1h, in agreement with previous studies [1] and indicating similar sized marginated and circulating intravascular pools. In contrast, the 45-minute recovery of eosinophils was 15 ± SEM 2%, suggesting a larger marginated or tissue pool. Moreover, they appeared to re-circulate at ~4 and 9h before monoexponential removal. Their mean intravascular residence time was 25.2 ± SEM 3.8h.

Conclusions: Eosinophils display intravascular kinetics strikingly different from neutrophils. Like lymphocytes, they re-circulate between intravascular and tissue pools.


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POSTER B-7

FINAL STAGES OF EOSINOPHIL DEVELOPMENT IS DRIVEN VIA AUTOCRINE STIMULATION

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Background: Eosinophils are derived in the bone marrow from a lineage-committed hematopoietic progenitor cell expressing CD34, IL-5Ra and low levels of c-kit. IL-5 is recognized as a critical regulator of eosinophilia with effects on bone marrow progenitors and mature eosinophils. IL-5-targeted therapy in experimental models and clinical trials results in a marked reduction in peripheral blood eosinophilia, but has been less effective in reducing mucosal tissue eosinophilia and has had little effect on eosinophil progenitors (EoPs) numbers in the bone marrow suggesting an unidentified alternative pathway promoting tissue eosinophilia and EoP survival.

Objective: To investigate the consequence of IL-5 neutralization on EoP differentiation.

Methods: Eosinophil differentiation was induced via ex vivo culturing of low density bone marrow cells from wild-type and IL-5-deficient mice with IL-5 for ten days with media changes every two days. For some cultures, IL-5 was not included in the media after day 4 and anti-IL-5 (clone # TRFK4) was added to neutralize IL-5 produced. EPO activity in total cell lysates was determined via colorimetric assay in a 96-well microtiter plate, through the addition of o-phenylenediamine-H2O2 buffer. Expression of surface Siglec-F was determined by flow cytometry. Cytokine levels in culture supernatants were measured via commercially available ELISA kits.

Results: We demonstrate that withdrawal of IL-5 after 4 days of stimulation (without addition of other cytokines) to developing eosinophils had no effect on the subsequent yield of mature eosinophils. In contrast, addition of an IL-5 neutralizing antibody after IL-5 withdrawal resulted in a 50% reduction in the production of mature eosinophils. Expression of surface Siglec-F on mature eosinophils and eosinophil peroxidase activity in lysates from mature eosinophils were augmented after IL-5 neutralization compared to eosinophils derived from cultures with IL-5 throughout the culture period. Developing eosinophils produced IL-33 and GM-CSF in the presence of IL-5 and, importantly, cytokine expression was maintained after IL-5 was withdrawn. Cultures of EoPs from IL-5-deficient bone marrow resulted in a ~30% reduction in yield of mature eosinophils after IL-5 withdrawal. Surprisingly, mature eosinophils derived from IL-5-deficient EoPs had markedly reduced (>50%) eosinophil peroxidase activity compared to eosinophils derived from wild-type EoPs, despite being cultured in exogenous IL-5.

Conclusions: These findings suggest that a brief exposure of EoPs to IL-5 is sufficient to initiate a differentiation program, including expression of eosinophil-promoting cytokines such as GM-CSF, IL-33 and IL-5 that sustains eosinophil development and does not require further IL-5 exposure. Further, our data indicates that the absence of IL-5 early in EoP generation results in sub-optimal granule production during eosinophil differentiation suggesting that there may be a difference in functional response between eosinophils produced in disease states with IL-5 stimulation and those produced at baseline. Together, these studies may mechanistically explain persistent tissue eosinophilia following IL-5-targeted therapy in patients and highlight the need for adjunct therapy to fully ablate eosinophils.
POSTER B-8

EFFECTS OF THYMIC STROMAL LYMPHOPOIETIN ON CORD BLOOD PROGENITOR CELL DIFFERENTIATION AND HEMOPOIETIC CYTOKINE RECEPTORS EXPRESSION

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Background/Objectives: TSLP has been described as a “master TH2 cytokine” with pleiotropic effects including the activation of cord blood (CB) CD34+ hematopoietic progenitor cells (HPC). Recent research shows that TSLP, combined with IL-25 and IL-33, induces a population of multi-potent mucosal progenitors in mice, recapitulating the longstanding observations on “in situ hemopoiesis” as a key element of allergic inflammation involving Eo/B progenitors in human airway. Recently, and relevantly, TSLP has been shown to directly activate MCs as well as CB CD34+ HPC, with release of proinflammatory TH2 cytokines and chemokines in a TSLP-dependent manner. Additionally, it has been suggested that TSLP may modulate the function of CD34+ cells via changes in hemopoietic cytokine receptors (HCR), which have been shown to be altered in CB CD34+ cells of atopic at-risk infants. In our current studies, we aim to characterize HCR expression patterns on CD34+ cells and to examine CB CD34+ cell differentiation following TSLP stimulation, with or without, IL-33 as a co-ligand.

Methods: Purified CD34+ progenitor cells were isolated from fresh and frozen CB using a progenitor cell enrichment kit via negative selection magnetic-activated cell sorting techniques. Isolated CD34+ cells were stimulated with varying doses of rTSLP (0.1, 0.5, and 1 ng/ml), with or without IL-33 then stained for surface expression of HCR, or used for methylcellulose colony assays. Flow cytometric staining of CB CD34+ cells were performed using monoclonal antibodies to CD34, CD45, polyclonal rabbit antibodies specific for IL-3Rα, IL-5Rα, and GM-CSFRα, and compared to isotype controls. Five thousand progenitors were collected for analysis, using a multi-parameter gating strategy. The specific mean fluorescence intensity (sMFI) was obtained by subtracting the MFI of the isotype control from the MFI of the receptor of interest. CD34+ cells were cultured in semisolid methylcellulose culture in the presence of optimal doses of recombinant cytokines, IL-3, IL-5, and GM-CSF. Cultures were performed in duplicates and incubated for 14 days. Mean numbers of Eo/B CFU were enumerated (colonies were defined as ≥40 cells).

Results/Conclusion: Overnight stimulation with TSLP (1 ng/mL) significantly enhanced mean expression of IL-5Rα on CB CD34+ cells (n=8, p=0.04). In addition, TSLP, with or without, IL-33 as a co-ligand was sufficient to induce the differentiation of CD34+ progenitors into eosinophils. Our findings suggest key roles for TSLP in influencing CB progenitor cell eosinophilic lineage commitment eventuating in allergic inflammation and disease in early life, and suggest TSLP as a novel therapeutic target in disease.

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EPISODE ANGIOEDEMA WITH EOSINOPHILIA (GLEICH’S SYNDROME) IS A CYTOKINE-MEDIATED, MULTILINEAGE CELL CYCLING DISORDER.

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Background: Gleich’s Syndrome is a rare disorder characterized by episodes of angioedema and eosinophilia that occur at 3-4 week intervals and resolve spontaneously without therapy. Early studies demonstrated elevation of serum IL-5 levels prior to the onset of eosinophilia. More recently, aberrant CD3-CD4+ lymphocyte populations have been detected in some patients, suggesting that Gleich’s syndrome may be a form of lymphocytic variant hypereosinophilic syndrome. This hypothesis is supported by the demonstration of eosinophil granule protein deposition in skin biopsies of patients during episodes of angioedema.

Objectives: To determine whether Gleich’s syndrome, like cyclic neutropenia, is a multilineage disorder and whether both eosinophil and non-eosinophil mechanisms play a role in the clinical manifestations of Gleich’s syndrome.

Methods: Three subjects with suspected Gleich’s syndrome were evaluated at the NIH. Complete blood counts and serum were collected from untreated subjects every 2-3 days over the course of 1-2 months and stored at -80 degrees. Serum IL-5, IL-8, eotaxin-1, GM-CSF, IFN-g, MIP-1-b, G-CSF, and IL-13 levels were assessed by suspension array technology in multiplex. Serum CCL17/TARC and tryptase were quantified by ELISA. Whole blood flow cytometry was performed to assess eosinophil activation, T cell phenotype, and to quantify T, B and NK subsets. T cell clonality was determined on the basis of TCR rearrangement patterns.

Results: Episodic eosinophilia and angioedema with a periodicity of 25 to 30 days was confirmed in all three subjects. Cycling of neutrophils, lymphocytes and platelets, but not monocytes, were also observed in all subjects. Peak neutrophil elevation (median 3.95 fold elevation) occurred prior to the peak of eosinophilia with return to baseline between cycles. An aberrant CD3-CD4+ T cell population was detected in all 3 subjects, and TCR rearrangement studies showed a clonal pattern in 2 subjects. Serial whole blood flow cytometry was performed in one subject and demonstrated cycling of the aberrant T cell population with the same timing as the eosinophilia. Peak serum levels of IL-5 (1019 pg/ml), GM-CSF (7.02 pg/ml), eotaxin-1 (86.1 pg/ml), IL-13 (114.6 pg/ml) and CCL17/TARC (64514.5pg/ml) were detected 5-7 days prior to maximal eosinophilia in one patient. Conversely, peak serum levels of inflammatory markers IL-1b (1.15 pg/ml) and MIP-1-b (371.6 pg/ml) were seen 2-7 days after maximal eosinophilia. Surface expression of activation markers CD69 and CD25 were increased on eosinophils from all subjects during symptoms and peaked prior to maximal eosinophilia in the one subject for whom serial measurements were available. Tryptase levels increased two-fold at the time of symptoms in one subject and returned to baseline 1 day prior to maximal eosinophilia. Skin biopsy performed during the symptomatic episode showed only occasional eosinophils and a moderate increase in mast cells.

Conclusions: Although the etiology of Gleich’s syndrome is not yet known, our data suggest that multiple lineages, including lymphocytes, neutrophils and mast cells, may be involved in disease pathogenesis. Whether these cells act directly or promote eosinophilia and eosinophil activation remains to be elucidated.

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POSTER B-10

ROLE OF IL-25 ON HUMAN BLOOD AND TISSUE EOSINOPHILS

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Background: Eosinophils recruited to epithelia barriers in response to allergens, or infectious agents, can be associated with exacerbation of Th2 mediated inflammation. IL-25 plays a major role in the initiation and maintain of allergic inflammation associated to eosinophils and Th2 cells (1). Eosinophils have been described as a major source of IL-25. Correlation between IL-25, eosinophilia and clinical features has been identified in Churg-Strauss Syndrome (CSS), associating asthma and massive eosinophilia (2). However, the direct effect of IL-25 on human eosinophils has never been studied.

Objectives: We hypothesize that human blood and tissue eosinophils can express IL-25R and may respond to IL-25 by releasing ROS and cationic proteins, leading to pro-inflammatory conditions.

Methods: Human peripheral blood eosinophils from healthy subjects and patient with Hyper Eosinophilic Syndrome (HES) were purified as previously described (3). Briefly, eosinophils were isolated from heparinized whole blood or tissues, using negative selection procedures (Miltenyi, MACS system). Tissue eosinophils were obtained from nasal polypes (NP) of patients suffering from Chronic Rhinosinusitis with nasal polyps (CRSwNP). For eosinophil purification from NP, we adapted a previously published protocol (4) optimizing enzymatic and mechanical digestion steps and cocktail antibodies for magnetic negative selection. Eosinophil purity was superior to 95% for blood samples and reached 75 to 90% for NP tissues. Flow cytometry analysis of IL-25R and IL-25 expression was performed on freshly purified eosinophils, in combination with detection of other membrane markers (HLA-DR, CD69, VLA4...). IL-25 mediated activation of eosinophils was further assessed by different approaches: measurement of Reactive Oxygen Species (ROS) and eosinophil specific granule proteins (ESGP) (EPO, ECP, EDN) release. Aliquots of culture supernatants were also collected for analysis of cytokine release. Aliquots of cells were collected when possible for further RNA isolation and total protein isolation for PCR and Western blots analyses.

Results: Flow cytometry analysis of freshly purified eosinophils revealed variable but constitutive membrane expression of IL-25 receptor on blood and tissue samples. This expression was associated with intracellular content of the protein. IL-25R membrane expression could not be correlated with the membrane expression of other markers tested. Stimulation of blood eosinophils with purified IL-25 (Peprotech) induced dose dependant production of Reactive Oxygen Species (ROS). Moreover overnight incubation of eosinophils with IL-25 could induce release of cationic proteins.

Conclusions: Here we showed that human eosinophils express IL25-R and that IL-25 could activate themselves, via an autocrine pathway, for releasing ROS and ESGPs. This result may contribute to a better understanding of interactions of eosinophils with major sources of IL-25 represented by activated epithelia and activated memory Th2 cells. Further experiments and extension of analysis to various clinical situations are needed to study complex modes of interaction between these different counterparts and to understand the pathogenesis and resolution of Th2 inflammatory response.


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POSTER B-11

IMATINIB MESYLATED IN HES ASSOCIATED OR NOT WITH FIP1L1-PDGFRα REARRANGEMENT: OVERVIEW OF 43 F/P+ CEL AND 20 F/P- HES

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1U995 Inserm ; Team 1,2; French Eosinophilic Network, EA2686, Laboratoire d'Immunologie ; 3Laboratoire d'Hématologie ; 4 Service de Médecine Interne, Centre Hospitalier Régional Universitaire de Lille, Université Lille-Nord de France, 59045 Lille Cedex, France; 5 Service d'Hématologie, CHU Angers, 49 000 Angers, France; 6 Université Versailles-Saint Quentin en Yvelines, Service d'Hématologie, Hôpital André Mignot, 78000 Versailles, France; 7 Service de Médecine Interne, CHU de Nantes, 44 000 Nantes, France; 8 Service d'hématologie, CHU Morvan, 5 avenue Foch, 29609 Brest; 9 Service d'Hématologie Clinique, CHU de Caen, Avenue de la Cote de Nacre, 14033 Caen Cedex, France; 10 Université Versailles-Saint Quentin en Yvelines, Hôpital Foch, Service de Médecine Interne, 40 rue Worth, 92151 Suresnes Cedex, France.

Background: The fusion gene FIP1L1-PDGFRα (F/P) is the most frequent clonal event identified in Hypereosinophilic Syndrome. Prognosis of F/P associated chronic eosinophilic leukemia (CEL) has been considerably improved by Imatinib mesylate (IM). Despite IM is recognized as treatment of choice for F/P+ CEL, regimen of IM remains controversial, concerning both the initial dose (100 to 400 mg/d) and the relevance of a weaker maintenance dosage. Moreover, the efficacy of IM for some F/P- HES patients, although already described, remains controversial, with variable hematologic remission rates in the literature (6-40%). In addition, any predictive factor of complete hematological response (CHR) with IM has been identified in such F/P- patients.

Objectives: To analyze, on the largest cohort of F/P+ CEL patients, use and efficiency of IM in order to bring newest insight concerning optimal dosing, duration of treatment and the possibility of definitive cure. To identify predictive factor of CHR in F/P- patients treated with IM. To evaluate the diagnosis interest of a surexpression of Wilms' tumor 1 (WT1) gene for discrimination of the HES variants.

Methods: A retrospective multicentric study coordinated by the French Eosinophil Network. Clinical and biological data were collected from 32 hospital centers after informed consent. In F/P+ CEL patient (n=43), analyzed parameters were CHR and complete molecular response (CMR); initial regimen, schedule of maintenance regimen. Efficiency of IM in F/P- HES patients (n=20) was evaluated by CHR. We studied correlation between CHR and clinical or biological characteristics before treatment with IM. WT1 expression (RQ-PCR) was evaluated in 3 defined group of HES patients (F/P+ CEL, lymphocytic HES [L-HES] and undefined HES [U-HES]), and also two control groups (reactive HE and hematological disorders [HD])

Results: CHR was obtained in 100% (43/43) and CMR in 97.1% of evaluated patients (33/34). Average initial dose of IM was of 166 mg/d. For 28 patients, IM dose was tapered slowly. This maintenance regimen (mean of 58 mg/d) allowed a sustained CHR. None developed resistance to IM, with a median follow up of 51.5 months (range 1.4–97.4). IM has been stopped for 5 patients without hematological or molecular relapse, after a median follow-up of 31 months (range 9-57). In our cohort of F/P- HES (n=20), IM induced a CHR in 33% of patients. This CHR was correlated positively with high B12 level and negatively with previous chronic HE to steroid. WT1 transcript amounts were significantly higher in F/P+ CEL (median: 1.212 %) and HD compared to L-HES (0.009%), U-HES (0.011%) or reactive HE (0.011%) but could not discriminate reactive HE from idiopathic HES or F/P+ CEL (p<0.0001).

Discussion: Not surprisingly, IM display a spectacular efficiency in F/P+ CEL, but our results suggest otherwise that a weak initial regimen and a lower maintenance regimen do not induce resistance, a major problem met in chronic myeloid leukemia (CML). Interestingly, stop of IM for 5 patients allowed long term - IM free - CHR and CMR. To our knowledge, this is the first report that IM may be curative in F/P+ CEL, as recently described in CML (1), while a previous prospective study described a relapse in all 5 F/P+ CEL patients after discontinuation of IM (2). Efficiency of IM in F/P- HES seems more inconstant and its use as second line could be guided notably by elevated rates of vitamin B12 and no response to a previous corticoid treatment. Analysis of WT1 transcript, a useful marker in monitoring AML residual disease, demonstrated that it should not be considered as a general marker of HES, contrary to previous report (3).

Conclusions: First, overview of 43 F/P+ CEL, the largest cohort described nowadays, demonstrated that low dose regimen IM (100 mg/d) induced CHR and CMR, but also may cure patients. Secondly, IM could be indicated for F/P- HES presenting compete resistance to steroids or elevated rates of vitamin B12. Finally, analyze of WT1 expression does not bring additional information in HES compared to cytogenetic, molecular and biochemical analysis already made in this context.


Grant Support: This work is funded by two national Programme Hospitalier de Recherche Clinique (PHRC) 2003 and 2008.
CONTRIBUTIONS OF NOTCH SIGNALLING TO GM-CSF-INDUCED EOSINOPHIL ERK1/2 ACTIVATION AND ENDOTHELIAL TRANSMIGRATION

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Background: Notch signaling is a fundamental pathway implicated in cell activation, differentiation and survival. We have previously reported that mature human eosinophils express Notch receptors and Notch ligands, and that Notch signaling is involved in GM-CSF-induced eosinophil polarization, chemokinesis and survival [1].

Objectives: Building upon our previous findings, the purposes of the current study are 1) To investigate the effect of Notch signaling on GM-CSF-mediated eosinophil ERK1/2 activation; and 2) To determine whether Notch signaling is required for GM-CSF-primed eosinophil transmigration across cytokine-activated endothelium.

Methods: Human eosinophils (> 99% purity) were isolated from allergic or normal blood donors by negative selection. Eosinophils were cultured in medium (RPMI1640 complete) alone, or containing GM-CSF (100 pM) with 10 μM of the Notch inhibitor GSI (g-secretase inhibitor II) or DMSO as a vehicle control for 2, 5, or 10 min, followed by immediate fixation and permeabilization. Expression of the cleaved Notch receptor intracellular domain (NICD) and ERK1/2 phosphorylation was determined by intracellular flow cytometric analysis. To perform eosinophil transmigration assays, human umbilical vein endothelial cells (HUVEC) were grown to confluence on transwell inserts, and overlaid with 0.2 X 10^6/well eosinophils. In some wells, endothelial cell monolayers were activated by IL-4/TNF-a (100 pM each) prior to transmigration. Eosinophils were cultured overnight in medium alone or primed with GM-CSF (1 pM) plus DMSO or GSI (10 μM). After transmigration, cells in the lower compartments were collected and counted by flow cytometry. Percent eosinophil transmigration was expressed as: (number of migrated eosinophils/total input eosinophils) X 100. Statistical significance was determined using paired Student’s t-tests.

Results: Intracellular NICD expression was detected in freshly isolated human eosinophils. Eosinophil phospho-ERK1/2 expression was induced by GM-CSF and significantly diminished in the presence of the Notch inhibitor GSI (p<0.05). GM-CSF-primed eosinophils demonstrated significantly increased transmigration through non-activated endothelial monolayers compared to non-primed eosinophils (p<0.05). The percentage of transmigrated eosinophils increased significantly in IL-4/TNF-a activated endothelial cell monolayers compared to non-activated monolayers with non-primed eosinophils (p<0.05, N=5). However, GM-CSF priming had no significant effect on eosinophil transmigration through activated endothelial cell monolayers. Inclusion of GSI significantly inhibited eosinophil transmigration in each condition compared to its DMSO control.

Conclusions: Our data has shown that human eosinophils constitutively express activated Notch receptor 1. Inhibition of Notch activation diminished GM-CSF-induced ERK1/2 phosphorylation in human eosinophils. Furthermore, GSI significantly inhibited eosinophil transmigration through HUVEC monolayers, indicating Notch signaling may play an important role in eosinophil transmigration.


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**POSTER B-13**

**CD34 LOCALIZATION IN EOSINOPHILS AT STEADY STATE AND DURING DISEASE**

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**Background:** The cell surface sialomucin CD34 has an emerging role in hematopoietic cell migration, notably on mast cells and eosinophils. Our research has demonstrated that CD34 surface expression promotes cell migration and CD34 ablation results in impaired homing and increased cell adhesion. As eosinophils are key inflammatory cells involved in the pathogenesis of allergic asthma and inflammatory bowel diseases (IBD), we assessed the effects of CD34 ablation in these diseases. In mouse models of both allergic asthma [1] and ulcerative colitis [2], CD34 ablation results in impaired eosinophil migration, resulting in attenuated disease pathology.

**Objectives:** In our current studies, we characterize eosinophil CD34 expression patterns at steady state and during disease pathology, to further explore the role of CD34 in eosinophil migration.

**Methods:** Asthma was induced using a standard OVA induction model [1], while ulcerative colitis was induced using 3.5% dextran sulfate sodium (DSS) [2]. In both disease models, mice were characterized to assess disease severity. Under disease conditions, CD34 expression was assessed using flow cytometry in digested lung and colon samples. At steady state, CD34 expression on the cell surface and in intracellular compartments was assessed on spleen and bone marrow cells isolated from mice with induced eosinophilia (pCD3-IL5Tg and pCD3-IL5TgCd34-/- strains).

**Results:** Cd34-/- mice exhibit decreased eosinophil infiltration and are resistant to both OVA-induced allergic asthma and DSS-induced ulcerative colitis. In allergic asthma, lung and alveolar eosinophils express moderate levels of surface CD34 and eosinophils purified from Cd34-/- lung tissues have decreased migration efficiency in vitro, compared to wildtype controls. In ulcerative colitis, approximately 30% of colon-infiltrating blood cells are eosinophils, which express high levels of surface CD34. At steady state, eosinophils purified from IL5Tg animals express low levels of surface CD34 within the blood, bone marrow and spleen, but high levels of surface CD34 expression in colon tissues. Intriguingly, permeabilization of steady-state spleen and marrow eosinophils reveals high levels of intracellular CD34 expression, when surface expression is absent.

**Conclusions:** Our findings suggest that surface CD34 expression is critical for optimal eosinophil migration during disease conditions and contributes to tissue pathology. In non-diseased tissues/conditions, surface CD34 expression is limited, but eosinophils exhibit high levels of intracellular expression. We hypothesize that intracellular CD34 may rapidly traffic to the cell surface upon eosinophil activation, as a novel mechanism regulating eosinophil migration.


**Grant Support:** This work is funded by the AllerGen NCE (Grant #3.14). SM received funding from the CIHR/HSF Transfusion Science Fellowships from the Centre for Blood Research (CBR) at UBC and MG received funding from the CIHR/MSFHR Transplantation Training Program. KMM is a MSFHR Scholar (Senior) and CBR Member.
ENDOTOXEMIA DECREASES EOSINOPHIL POTENTIAL IN BONE MARROW

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**Background:** Eosinophilia is a common clinical problem associated with atopic disease and parasitic infection. In contrast, a marked decrease in circulating eosinophils, or eosinopenia, has been demonstrated to be a marker of acute bacterial infection and has been shown to have value in the diagnosis of sepsis in the ICU setting. The molecular mechanism of eosinopenia associated with bacterial infection has not been elucidated.

**Objective:** To investigate the consequence of exposure to systemic bacterial lipopolysaccharide (LPS) on eosinophil progenitors in the bone marrow.

**Methods:** Endotoxemia was induced via intraperitoneal administration of LPS (Sigma, 10mg, 25mg, and 40mg) to wild-type mice (BALB/c) or mice with marked hypereosinophilia (CD2-IL-5 transgenic mice). Low density bone marrow (LDBM) cells were harvested 24 hours or one week later. Eosinophil progenitor (EoP) numbers were measured indirectly via eosinophil colony assay with LDBM cells plated in methylcellulose-based medium containing either IL-5 (Peprotech) alone or the cytokine cocktail of IL-5, GM-CSF (Peprotech) and IL-3 (Peprotech). Colonies on each plate were counted following 7-10 days, normalized to number of cells plated in each dish, and reported as colonies formed (CFU) per 10,000 cells plated. Blood was collected by retro-orbital bleed in EDTA tubes and then fixed with paraformaldehyde prior to staining for surface markers. Expression of surface markers was determined by flow cytometry.

**Results:** TLR4 protein expression was confirmed on the surface of CD34-expressing progenitors in the bone marrow of wild-type mice. Consistent with previous studies, mature eosinophils were negative for surface expression of TLR4. Endotoxemia resulted in significant and specific reduction in a dose-dependent manner in the number of eosinophil colonies formed from LDBM cells (57% and 80% reduction following 10ug and 40ug LPS treatment, respectively) compared to saline-treated controls. In contrast, LPS treatment resulted in a modest increase in myeloid colonies formed containing neutrophils, eosinophils and macrophages. Mature eosinophils found in the blood of IL-5 transgenic mice were significantly reduced four days after LPS treatment (7±5%, n=8 mice) when compared to saline-treated (17±5%, n=8 mice) controls. The decrease in eosinophil progenitors in the bone marrow was still present eight days after induction of endotoxemia in wild-type mice. While the number of EoPs was decreased following endotoxemia, the number of mature eosinophils in the bone marrow remained unchanged.

**Conclusion:** Together, these findings suggest a direct effect of LPS via TLR4 on eosinophil progenitors to inhibit progenitor accumulation as an explanation for eosinopenia following serious bacterial infections. Our data also demonstrate a differential sensitivity to LPS between EoPs and mature eosinophils.
POSTER B-15
A WEEK – OR MORE? – IN THE LIFE OF A HUMAN EOSINOPHIL

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Background: Allergic asthma is accompanied by chronic inflammation in the airways. This inflammation is characterized by the presence of eosinophils and CD4+ T cells. Evidence suggests that this inflammation is important in allergic asthma. Despite numerous studies focussed on the pathogenesis of allergic asthma, some very basic issues remain to be elucidated. One important caveat is the lack of knowledge regarding the kinetics of inflammatory effector cells during normal immune homeostasis and under (allergic) diseased conditions, both in blood and tissue.

A good example for this is the seemingly contradictory findings from anti-IL-5 (mepoluzimab) trials for treatment of eosinophil asthma. In these trials, anti-IL-5 treatment led to a quick resolution of eosinophils in blood and sputum (1). After 20 weeks of treatment, however, eosinophils were still present in the lungs (2). Only after 50 weeks anti-IL-5 treatment decreased the clinical severity of a certain phenotype of asthma associated with eosinophil bronchitis (3). It is difficult to reconcile these studies with a short lifespan of eosinophils.

Objectives: To determine the half-life of granulocytes in peripheral blood and tissues in human using in vivo deuterium (2H) labeling.

Methods: We will apply a technique that allows the study of untouched granulocytes in humans (4). Healthy volunteers will take oral doses of 2H2O or 2H-glucose, which is incorporated in the DNA of all dividing cells. Hereafter, small blood samples will be taken and different cell populations will be obtained by high performance cell sorting. DNA of these cells will be isolated and its relative content of 2H/1H determined by a combination of gas-chromatography and mass spectrometry. In combination with mathematical modelling, this technique allows for determination of life spans of cells in peripheral blood. To study the half lives of eosinophils and neutrophils in tissue we will use the same technology, but with granulocytes isolated from induced sputum before and after allergen challenge.

Results: We have shown a neutrophil half life of 2.75 days and a lifespan of 5.4 days in human peripheral blood, over ten times longer than previously reported (5). Preliminary data on the half life of eosinophils in human peripheral blood will be available at the time of the 7th IES conference.

References:

Grant Support: This work is funded by the Dutch Lung Foundation, grant #10.052
POSTER B-16
MATERNAL ALLERGY MODULATES CORD BLOOD HEMOPOIETIC PROGENITOR TOLL-LIKE RECEPTOR EXPRESSION AND FUNCTION

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Background: Little is known regarding the prenatal determinants of innate immune responses in relation to infant allergic risk. Environmental exposures, including microbial stimuli, may predispose susceptible individuals to atopy and asthma in early infancy or even in utero.

Objective: Since Toll-like receptors (TLRs) recognize microbial products, and since cord blood (CB) progenitor alterations have been observed in neonates at risk for atopy, we investigated the expression and function of TLRs on CB hemopoietic progenitors, in relation to atopic risk as defined by maternal allergic sensitization.

Methods: Thirty-two (15 low- and 17 high-atopic risk) infant CB samples were assessed for phenotypic and functional alterations in CD34+ cells by flow cytometry and methylcellulose cultures, respectively. CD34+ hemopoietic progenitors were stained for TLR-2, -4, -9, GM-CSFRα, IL-5Rα and IL-3Rα, or cultured in methylcellulose assays for hemopoietic cytokine-stimulated eosinophil-basophil (Eo/B) colony forming units (CFU), with or without lipopolysaccharide (LPS).

Results: High-atopic risk infants had significantly lower CB CD34+ cell TLR-2, TLR-4 and TLR-9 expression (p=0.009). High risk infant progenitors gave rise to significantly more Eo/B CFUs (p=0.002) upon hemopoietic cytokine (IL-3, IL-5 or GM-CSF) stimulation ex vivo. While LPS co-stimulation induced Eo/B CFUs from both low- and high-risk infant CB CD34+ cells, this response was significantly (p=0.020) muted in the high-risk CB progenitors.

Conclusions: Neonatal CB CD34+ hemopoietic progenitor cell TLR expression and functional responsiveness are altered in CB from atopic at-risk infants. Maternal allergic sensitization may modulate hemopoietic progenitor TLR expression and function in utero; specifically, Eo/B ‘lineage priming’ at birth may be circumvented through engagement of TLR pathways in early life.

Grant Support: AllerGen N.C.E and CIHR
CHARACTERIZATION OF A NOVEL HUMAN HEMATOPOIETIC PROTEIN, TMEM103.

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Background: The myriad of eosinophil related diseases has led us to investigate eosinophil development in an attempt to understand the signals prompting eosinophil production in bone marrow. Gene profiling of developing eosinophils has shown that a novel putative protein, TMEM103, is expressed as mRNA. The TMEM103 open reading frame is conserved in mammals, chicken and stickleback fish.

Objectives: We hypothesize that TMEM103 is expressed during myeloid development, following commitment of the common myeloid progenitor and may be involved in eosinophil and monocyte development.

Methods: Human bone marrow mononuclear cells were purchased from Allcells.com and anti-TMEM103 was labeled with FITC for use in flow cytometry and immunofluorescence. Cells were sorted with a Becton Dickinson FACS Aria II and cytopspun onto slides for microscopy or grown in methylcellulose with SCF, Flt-3 ligand, IL-3, IL-5 and epoietin-α. Human protein lysates for western blot were purchased from Biochain and western blots were performed and analyzed on a LICOR system using infrared labeled secondary probes.

Results: A monoclonal antibody was raised to recombinant human TMEM103 and the existence of the approximately 30 kDa native protein in several tissues has been confirmed by western blot analysis. TMEM103 is expressed on the surface of a subset of bone marrow cells as shown by immunofluorescence and flow cytometry. TMEM103+ cells also express myeloid markers such as CD33, CD7, CD13 and CD15 but not markers of primitive hematopoietic cells such as CD34 and CD117. Sorted TMEM103+ cells are mainly developing monocytes and granulocytes as shown by microscopy, whereas TMEM103- cells are mainly erythrocytes. In addition, sorted TMEM103+ cells grown in semisolid media grow into macrophages and as yet undetermined white cell colonies, whereas TMEM103- cells grown under the same conditions develop into erythrocytic colonies. Future experiments involve RNA silencing of TMEM103 to determine its function in hematopoiesis.

Conclusions: TMEM103 is expressed in the bone marrow and may be functionally involved in myeloid development.

Grant support: This work was supported by an R21 grant from NIH and the University of Utah Foundation.
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POSTER C-1

CD103 IN THE CONTROL OF PULMONARY EOSINOPHILIA IN ASTHMA

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Background: CD103 (or alpha-E integrin) is expressed on a subset of intraepithelial dendritic cells (DCs) and T regulatory cells. CD103+ DCs are known to drive a regulatory response via the differentiation of T naive cells into T regulatory cells. However, the exact role of CD103 in the development of a complex inflammatory reaction such as asthma is still undefined.

Objectives: We set out to verify whether CD103 plays a role in the development of allergic asthma and eosinophil recruitment to the lung, using a well-described mouse model of asthma developed in Cd103-/- mice and C57Bl/6 wild type mice.

Methods: For the induction of asthma, wild type C57Bl/6 and Cd103-/- mice were injected intraperitoneally on days 1 and 8 with 0.2% ovalbumin (OVA) coupled with Al(OH)3. 14 days later, mice were challenged intranasally with 2% OVA for five days over a period of 7 days. The total pulmonary inflammation as well as eosinophilia was evaluated via the analysis of the broncho-alveolar lavage (BAL) content as well as the inflammatory cell content of collagenase-digested lung preparations. Histology sections were evaluated for peribronchial and perivascular infiltration of the lung tissue, and airway resistance was tested using the FlexiVent apparatus. Ex-vivo OVA-specific cytokine production of lung inflammatory cells was evaluated by cytometric bead array and serum OVA-specific IgE and IgG1 were quantified.

Results: Surprisingly, in this mouse model of asthma, Cd103-/- mice developed an exacerbated pulmonary inflammatory response compared to wild type mice. Indeed, total inflammatory cells as well as eosinophil numbers and % were significantly increased in CD103-deficient mice, with eosinophil percentages up to 75% in the BAL of Cd103-/- mice. Also, when histological sections were evaluated, Cd103-/- mice demonstrated significantly higher levels of peribronchial and parenchymal infiltration compared to wild type mice. Moreover, the increase in airway resistance in response to metacholine was higher in mice lacking CD103 expression compared to wild type. Finally, the production of IFN-gamma, TNF, IL-10 and MCP1 by total lung inflammatory cells was higher in cells isolated from Cd103-/- mice compared to wild type, whereas the levels of serum OVA-specific IgE and IgG1 were lower in mice lacking CD103 expression.

Conclusions: We conclude that CD103 plays a pivotal role in the control of the inflammatory response in this mouse model of asthma. Mechanisms behind this exacerbated phenotype are yet to be determined.

Grant Support: This work is funded by The AllerGen Network for Excellence (KMM), The IUCPQ JD Begin Foundation (MRB) as well as the CRIUCPQ (MRB).
SELECTIVE BLOCKADE OF M3 MUSCARINIC RECEPTORS WITH TIOTROPIUM REDUCES EOSINOPHILS AND PREVENTS AIRWAY HYPERREACTIVITY FOLLOWING ANTIGEN CHALLENGE

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Background: Asthma is characterized by reversible periods of bronchoconstriction, inflammation, and airway hyperreactivity. Bronchoconstriction is mediated by acetylcholine stimulation of M3 muscarinic receptors on airway smooth muscle. Muscarinic receptor antagonists are not very effective when used to treat chronic asthma. This may be because currently available muscarinic receptor antagonists are not selective for M3 receptors over other muscarinic receptor subtypes in the lung. Since M3 muscarinic receptors are expressed by inflammatory cells, including eosinophils, which are important in the development of airway hyperreactivity following allergen exposure we tested whether selective blockade of M3 receptors could be beneficial in asthma.

Objectives: To determine whether selective blockade of M3 receptors, at the time of antigen challenge, blocks development of airway hyperreactivity and inflammation in antigen challenged guinea pigs.

Methods: Hartley guinea pigs (150-200g) were sensitized with i.p. ovalbumin on days 1, 3, and 6. Three weeks later, sensitized guinea pigs were pretreated with either lactose vehicle or tiotropium bromide (1 mg/kg), a kinetically selective M3 receptor antagonist, and then challenged with inhaled ovalbumin (5% in PBS, for 10 min). 24 h after challenge bronchoconstriction was measured in anesthetized guinea pigs. Bronchoconstriction was measured as an increase in pulmonary inflation pressure in response to electrical stimulation of the vagus nerves (neuronally released acetylcholine, 10V, 0.2 ms, 2-25 Hz) or intravenous acetylcholine (0.1-10 mg/kg). Eosinophil density in airway smooth muscle and their association with nerves were quantified in tissue sections stained with an antibody against PGP9.5 (neuronal marker) and chromotrope 2R (eosinophil marker).

Results: Electrical stimulation of both vagus nerves induced frequency dependent bronchoconstriction that was significantly increased after antigen challenge. Selectively blocking M3 receptors with tiotropium at the time of challenge completely blocked antigen induced hyperreactivity. Tiotropium did not, however, inhibit vagally induced bronchoconstriction in sensitized (not challenged) control guinea pigs. These data suggest that tiotropium prevents airway hyperreactivity via a mechanism that is separate from inhibition of bronchoconstriction. Tissue eosinophils were significantly increased in airway smooth muscle and around nerves in lactose pretreated guinea pigs following challenge. Tiotropium pretreatment reduced this eosinophil accumulation following antigen challenge, suggesting that tiotropium may prevent airway hyperreactivity through an anti-inflammatory mechanism.

Conclusions: Our data suggest that selective blockade of M3 receptors may be an effective treatment for patients with eosinophilic asthma separate from inhibition of bronchoconstriction.

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POSTER C-3
IMMUNOREgULATION OF EOSINOPHILs BY CALCITRIOL

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**Background:** The prevalence of allergic diseases and asthma has increased worldwide at least during the last 3 decades. Meanwhile the incidence of various autoimmune and allergic conditions appears to be higher further away from equator. Amongst the hypothesis suggested to connect these observations is the lack of exposure to sufficient sunlight and therefore relative vitamin D deficiency. The epidemiologic link between vitamin D deficiency and asthma is still equivocal; some epidemiologic studies even associated maternal high doses of vitamin D supplementation with increased incidence of wheezing and atopy in children. Although, nothing is known about the possible direct activity of vitamin D on eosinophils, an inflammatory cell implicated at least in the chronicity of allergic asthma.

Hypothesis: Calcitriol (1α,25-(OH)2D3) exert regulatory effect directly on eosinophils biological activities possibly implicated in the immune features of allergic asthma.

**Methods:** Blood eosinophils from atopic donors were isolated and incubated over a 72h period with physiological concentrations of calcitriol (0 to 100 nM). Annexin V-PI flow cytometry assay was used to measure apoptosis.

**Results:** Preliminary results indicate that increasing concentration of calcitriol are able to sustain the viability of blood eosinophils *in vitro* without the addition of any other anti-apoptotic factors (n = 6, p < 0.05).

**Conclusion:** Our preliminary data on sustained viability suggest that calcitriol is a potent immune regulator of eosinophil function. We hope to unveil some other activities of this vitamin on eosinophils and perhaps explain further the complex physiopathology of allergic asthma.
**POSTER C-4**

**EOSINOPHIL INTEGRIN EXPRESSION AND ACTIVATION IN BLOOD AND AIRWAY PRE- AND POST-SEGMENTAL LUNG ANTIGEN CHALLENGE AFTER AND BEFORE ANTI-INTERLEUKIN-5 (MEPOLIZUMAB) ADMINISTRATION**

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**Background:** Integrins mediate cell adhesion to matrix proteins and other cells influencing cell signaling, activation, and migration. Integrins exist in bent inactive and various extended active conformations; activation can be monitored by conformation-specific monoclonal antibodies (mAbs)[1]. We reported that following segmental antigen challenge, patients with mild asthma have activated b1-integrins on eosinophils in blood and bronchoalveolar lavage (BAL) as assessed by mAb N29 and activation of b2-integrins in BAL but not in blood as assessed by mAb24 [2].

**Objectives:** To determine the role of IL-5 in b1- and b2-integrin activation by examining blood and BAL eosinophils obtained pre- and post-segmental lung antigen challenge from subjects with mild allergic asthma studied at baseline and repeated after administration of anti-interleukin(IL)-5 (mepolizumab).

**Methods:** Blood at 0, 2, or 48 h and BAL at 48 h post-challenge from 9 subjects with mild asthma before and after anti-IL-5 administration were analyzed by flow cytometry; using an expanded panel of 9 antibodies: MAR4, total b1-integrins; N29 and 8E3, intermediate and high activity b1-integrins; HUTS-21 and 9EG7, high activity b1-integrins; L130, total b2-integrins; KIM-127, intermediate and high activity b2-integrins; mAb24, high activity b2-integrins; LM11, total aMb2 integrin; and CBRM1/5, high activity aMb2 integrin.

**Results:** Prior to treatment with anti-IL-5, blood eosinophils had increases in median values of 1.2-fold for total b2, 1.1-fold for total aMb2, and 2.0-fold for KIM-127-reactive b2 48 h post-segmental antigen challenge compared to pre-challenge. These changes were not seen when the studies were repeated after administration of anti-IL-5. Compared to eosinophils from pre-challenge blood, eosinophils from BAL obtained 48 h post-challenge had increased expression and activation of b2 and aMb2 (1.5-fold for total b2, 1.4-fold for total aMb2, 8.4-fold for KIM-127-reactive b2, and 8.2-fold for CBRM1/5-reactive aMb2). Similar changes in b2-integrins on BAL eosinophils occurred after administration of anti-IL-5. In contrast to the results with b2-integrins on blood eosinophils, activation of eosinophil b1-integrins in blood and BAL after segmental antigen challenge was similar prior to and following administration of anti-IL-5.

**Conclusions:** There is limited IL-5-dependent upregulation of aMb2 and adoption of the intermediate activated b2 conformation on blood eosinophils after segmental antigen challenge, presumably due to exposure to IL-5 in the bone marrow and circulation. IL-5, however, does not seem necessary for more marked upregulation and activation of surface b2-integrins, including aMb2, on eosinophils in the airway. IL-3 and GM-CSF likely compensate for lack of IL-5 to modulate b2-integrins on airway eosinophils. In contrast, b1 activation on blood and airway eosinophils appears to be IL-5-independent. These results are compatible with our recently published *in vitro* data showing that IL-5 activates b2 but not b1 whereas P-selectin activates b1 but not b2 [3].

**References:**

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**POSTER C-5**

**UTILIZATION OF SEGMENTAL BRONCHOPROVOCATION TO STUDY THE EFFECT OF ANTI-IL-5 (MEPOLIZUMAB) ON EOSINOPHIL PHENOTYPE AND RECRUITMENT TO THE AIRWAY IN SUBJECTS WITH ATOPIC ASTHMA**


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**Background:** IL-5 plays a pivotal role in eosinophil maturation, migration and priming. Consequently, IL-5-blocking antibodies diminish circulating eosinophils and have been utilized with varying degrees of success as therapeutic agents in eosinophil-mediated diseases. The limited therapeutic effects may be due in part to persistence of preexisting eosinophils in the tissues or recruitment of a unique population of eosinophils that are IL-5-independent.

**Objectives:** To determine the role of anti-IL-5 in modulating eosinophil phenotype and recruitment to the airway we undertook the unique approach of segmental bronchoprovocation (SBP) with aeroallergen (Ag) to induce an intense, localized airway eosinophilia in subjects with mild allergic asthma. This model allowed us to study Ag-induced activation and recruitment of eosinophils in the absence of concurrent in vivo exposure to IL-5.

**Methods:** Nine subjects with atopic asthma underwent SBP-Ag before and one month after administration of anti-IL-5 (mepolizumab, 750 mg iv). Flow cytometric analysis of eosinophils was performed utilizing EDTA-treated whole blood and unseparated BAL cells obtained 48 h after SBP-Ag. Eosinophils were also purified from blood and BAL for assessment by quantitative real-time PCR (qPCR).

**Results:** Forty-eight hours after SBP-Ag, BAL eosinophils increased from <1% at baseline to 71±8% (mean ± SD) and total numbers of BAL cells from 13±6 to 177±156 X 104/ml BAL fluid. Circulating eosinophils decreased within one week from administration of mepolizumab and remained below 100 per mm2 for at least 2 months. However, the percentage of BAL eosinophil increased from <1 to 31±14 % and total numbers of BAL cells from 14±10 to 72±55 X104/ml BAL fluid in response to Ag challenge. Circulating eosinophils expressed high levels of IL-5 and GM-CSF receptors that did not change after administration of mepolizumab. Conversely, there was a striking decrease in IL-3Rα as assessed by flow cytometry and by qPCR. The IL-5-responsive markers, CD11b and CD66e were also significantly reduced after mepolizumab; however, CD69, CD23, and CCR3 were not changed. In contrast to blood, BAL eosinophils retained high levels of IL-3Rα and CD11b. Furthermore, IL-5Rα, which we previously demonstrated to be downregulated by IL-5, was nearly undetectable on BAL eosinophils and did not increase after mepolizumab.

**Conclusions:** These data suggest that in the circulation, eosinophil expression of IL-3Rα, CD11b, and CD66e is regulated by IL-5 and that anti-IL-5 treatment may alter the eosinophil’s ability to response to IL-3. The lack of phenotypic changes in BAL eosinophils and the substantial recruitment of eosinophils to the airway despite the diminution of circulating eosinophils raise questions concerning the origin of AG-induced BAL eosinophil. While it is possible that even with low levels of circulating eosinophil there are still sufficient numbers available for recruitment to the airway, it is also likely that allergen challenge induces expansion/differentiation of resident eosinophil progenitor cells. Analysis of archived biopsy specimens and qPCR of purified BAL eosinophils will allow us to determine the presence of SBP-Ag-induced eosinophil progenitors before and after mepolizumab.

**Grant Support:** NIH P01 HL88594. Mepolizumab provided by Glaxo Smith Kline.
POSTER C-6
PARTIAL CHARACTERIZATION OF THE EOSINOPHIL SIGLEC-F GLYCAN LIGAND PRODUCED BY MOUSE AIRWAY EPITHELIAL CELLS
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Background: Siglec-F, an inhibitory receptor on mouse eosinophils, is thought to bind 6'-sulfated sialyl Lewis X-containing structures constitutively expressed on airway epithelium that are enhanced during Th2 inflammation, but little is known about the regulation of Siglec-F ligand synthesis.

Objectives: We hypothesize that primary airway epithelial cultures will express sialidase-sensitive Siglec-F ligands and stimulation with Th2 cytokines (IL-4 or IL-13) will enhance Siglec-F ligand expression. We also hypothesize that expression of the specific posttranslational enzymes, namely the sulfo- and sialyltransferases KSGal6ST & St3gal3, will be increased when Siglec-F ligand expression is increased by stimulation, as these enzymes are putatively necessary for synthesis of the Siglec-F glycan ligand.

Methods: Primary mouse tracheal epithelial cells (mTEC) were isolated as described [1]. In some experiments, cells were stimulated with either IL-4 or IL-13 (50 ng/ml) for 4 hr or 24 hr then harvested. Expression levels of Siglec-F binding proteins, as well as the KSGal6ST and St3gal3 enzymes, were compared in western blot assays. The membranes were incubated with Siglec-F-human IgG1 Fc chimera or each primary anti-enzyme Ab, then incubated with appropriate secondary Ab. Bands were visualized using the ECL western blotting detection system. Expression of ligands by mTEC was studied via immunocytochemistry using Siglec-F/Fc or an isotype-matched control antibody (both at 1 μg/ml, 1 hr, 37°C), then incubated with secondary biotinylated polyclonal anti-human IgG antibody. Samples were then washed and incubated with streptavidin/alkaline phosphatase linker, followed by visualization with Vector red chromogen. In some experiments, samples were pretreated with or without sialidase (Clostridium sialidase, 0.02 U/sample, 2 hr). Permeabilization was performed in some experiments along with flow cytometric analysis to determine intracellular and cell surface expression levels of Siglec-F ligands. Harvested mTEC single cell suspensions were fixed in 4% paraformaldehyde (5 min, RT). To permeabilize, samples were incubated in PBS-S (0.1% BSA + 0.1% saponin + 1 mM Ca2+Mg2+/PBS, 10min, 4°C) before staining with Siglec-F/Fc or isotype-matched negative control. After washing, the samples were incubated with appropriate secondary Ab then analyzed by flow cytometry.

Results: A Siglec-F/Fc binding, sialidase-sensitive band (≈225 kDa) was detected in lysates by Western blot. In immunocytochemical staining, there was a sizable intracellular pool of this ligand. IL-4 and IL-13 stimulation enhanced Siglec-F ligand protein expression in mTEC lysates. Furthermore, a Siglec-F/Fc binding, sialidase-sensitive band (≈460 kDa) was detected in mTEC supernatants by Western blot and was increased by IL-4, indicating that this Siglec-F ligand is secreted. There did not appear to be a correlation between cytokine enhancement of ligand levels and levels of either KSGal6ST or St3gal3 protein, although IL-4 and IL-13 induced mRNA levels.

Conclusions: mTEC, especially after IL-4 treatment, make and secrete a high molecular weight sialylated Siglec-F ligand and appear well suited for subsequent studies whose goal is to isolate enough Siglec-F ligand for its full structural characterization.


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REGULATION OF THE DOUBLE-EDGE SWORD EFFECTS OF EOSINOPHILS IN MYCOPLASMA EXACERBATIONS OF ALLERGIC AIRWAY DISEASE BY SURFACTANT PROTEIN-A.

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**Background:** Surfactant protein-A (SP-A) has well-established functions in reducing viral and bacterial infections but its role in chronic lung disease, such as asthma, is less well-defined. Mycoplasma pneumoniae (Mp) frequently colonizes the airways of many chronic asthmatics and is thought to contribute to exacerbations of symptoms. Our lab has previously reported that in Ova-allergic mice SP-A deficiency leads to dramatic increases in eosinophilic infiltration and inflammation of the airway (1). Additionally, SP-A null mice have enhanced airway hyperresponsiveness (AHR), in response to Mp infection alone (2).

**Objective:** The research goal was to test the hypothesis that SP-A can also prevent Mp-induced degranulation of eosinophils and thereby protect the airways from injury due to release of inflammatory mediators.

**Methods:** *In vivo* experiments were conducted in wild type (WT) and SP-A null mice challenged with the model antigen ovalbumin (Ova) or Ova and Mp (Ova+Mp). Airway physiology measurements to a methacholine provocation were conducted 72 hours post-infection using Flexivent technology. *In vitro* experiments were conducted with purified eosinophils isolated from the blood of IL-5 transgenic mice and with human SP-A isolated from pulmonary alveolar proteinosis (PAP) patients.

**Results:** As previously reported, when Mp infects an allergic airway (Ova+Mp), AHR is greatly enhanced as compared to WT allergic (Ova only) mice (3). While mice lacking SP-A exhibit a significant augmentation of AHR and inflammation during concurrent allergic airway disease and infection (Ova+Mp) as compared to the WT mice of the same treatment group, they have significantly decreased Mp burden. Eosinophil mediators, such as eosinophil peroxidase (EPO) and eosinophil ribonuclease, that are implicated in pathogen killing mechanisms and in epithelial dysfunction are enhanced in samples from allergic/infected SP-A null mice as compared to WT mice, further suggesting an essential regulatory role for SP-A in this allergic/infectious model. *In vitro* findings suggest that SP-A functions by limiting the release of EPO from Mp-stimulated eosinophils, and thereby reducing their killing capacity for Mp.

**Conclusions:** SP-A appears to interfere with eosinophil-mediated biologic clearance of Mp by modulating the interaction of Mp with eosinophils, while simultaneously benefiting airway homeostasis by controlling eosinophil activation and release of harmful eosinophilic factors that can lead to airway constriction.

**Acknowledgements:** IL-5 transgenic mice were generated by Dr. James Lee.

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**References:**
CHARACTERIZATION OF OVALBUMIN-INDUCED EOSINOPHILIC INFLAMMATION IN LEPTIN-DEFICIENT MICE

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Background: Studies indicate that obesity increases the prevalence and incidence of asthma, and reduces asthma control. Leptin is a satiety hormone that modulates some of the proinflammatory effects observed in obesity. Leptin-deficient mice (ob/ob mice) show innate airway hyperresponsiveness and elevated responsiveness to methacholine following allergen sensitization and challenge [2,3]. Ovalbumin (OVA) challenge in high-fat fed mice promoted a markedly higher eosinophil (EO) accumulation in bone marrow and connective tissue surrounding the bronchial and bronchiolar segments [4].

Objective: The aim of this study was to investigate the effects of OVA sensitization and challenge on EO lineage in ob/ob mice.

Methods: All animal procedures and the experimental protocols were approved by the Ethical Principles in Animal Research. Wild-type (WT) and ob-ob mice were sensitized with two s.c. OVA injections mixed with Al(OH)3 at 7-day intervals. Mice were intranasally challenged with OVA twice a day. At 24-72 h after OVA challenge, EO counts in blood, bronchoalveolar lavage fluid (BALF) and bone marrow (BM) were carried out. Lungs were collected for histological analysis, and Th2 cytokine levels were measured in BALF.

Results: In WT mice, intranasal challenge with OVA significantly increased (p<0.05) the EO counts in BALF, reaching the peak at 48 h (0.31±0.3 and 0.007±0.004 x106/BALF, for OVA and PBS, respectively). In ob/ob mice, intranasal challenge with OVA also significantly increased the EO counts in BALF in all studied time (72 h: 0.4±0.06 and 0.006±0.002 x106/BAL, for OVA and PBS respectively, n=9-14). The histological data showed a 4.4-fold increase in EO counts in lung tissue of ob/ob mice compared with WT mice (p<0.05). At 72 h post-OVA challenge, EO counts were greater in lung tissue of ob/ob mice compared with PBS-challenged and WT OVA-challenged mice (80.0±3, 20.4±1.5 and 41.7±1.5 eosinophils/μm2, respectively; n=6 each). The BM counts of mature EO in OVA-challenged mice of ob/ob mice were significantly higher compared with WT mice (72 h: 1.5±0.14 and 1.1±0.1 x106/mL, respectively, n=6). The EO counts in blood in sensitized mice (48 h) were greater in ob/ob mice compared with WT mice. In ob/ob mice, the TNF-α (p<0.05) and IL-10 (p<0.0001) levels were greater, whereas the IL-6 (p<0.05) levels lower compared with WT mice. The IL-5 and eotaxin levels did not differ between groups.

Conclusions: We showed that obesity increases EO counts in lung parenchyma, and enhances the allergic eosinophilic influx to lung by mechanisms involving up-regulation of TNF-α and IL-10.

References:

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POSTER C-9

EOSINOPHILS SUPPRESS EPITHELIAL CELL INTERFERON EXPRESSION

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Background: Airway eosinophilia is a risk factor for virus-induced wheezing and exacerbations of asthma.

Objectives: We sought to determine whether eosinophils inhibit the epithelial cell antiviral response to human rhinovirus (HRV) infection.

Methods: We utilized an in vitro cell culture model consisting of BEAS-2B epithelial cell line, primary human eosinophils, and poly-IC or HRV-1A. BEAS-2B cells were cultured for 24 - 48 hours and poly-IC (25 µg/mL) or HRV-1A (20 MOI) was added with or without eosinophils (10⁶ per mL) for an additional 24 hours. Transwell plates were used to separate the eosinophils and BEAS-2B cells. A pharmacological inhibitor (SB 431542) of the activin receptor-like kinase (ALK5) was added to the culture to inhibit TGF-β signaling. Supernatants were collected for ELISA and RNA was prepared from BEAS-2B cells for quantitative PCR of IFN-β1 and IFN-λ1.

Results: Both poly-IC and HRV-1A induced BEAS-2B expression of IFN-β1 and IFN-λ1 mRNA. The addition of eosinophils resulted in a 74% suppression of poly-IC induced IFN-λ1 (p=0.023) and a tendency for 30% suppression of IFN-β1 (p=0.11). Eosinophils also suppressed HRV-1A induced IFN-β1 (83%, p=0.002) and IFN-λ1 (80%, p=0.002). Separation of eosinophils from the BEAS-2B cells in transwell plates did not affect the suppression. TGF-β protein concentrations were elevated in the cultures containing eosinophils. Inhibition of TGF-β signaling abrogated eosinophil mediated suppression of IFN-β1 and IFN-λ1.

Conclusions: Eosinophil release of TGFβ suppresses HRV-induced epithelial cell expression of IFN-β1 and IFN-λ1. Since these interferons have antiviral effects, this may represent a mechanism by which airway eosinophilia promotes virus-induced exacerbations of asthma.

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POSTER C-10
IMMUNODETECTION OF OCCULT EOSINOPHILS IN LUNG TISSUE BIOPSIES MAY HELP PREDICT SURVIVAL IN ACUTE LUNG INJURY

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Background: Acute lung injury (ALI) is a serious respiratory disorder for which therapy is primarily supportive once infection is excluded. Surgical lung biopsy may rule out other diagnoses, but has not been generally useful for therapy decisions or prognosis in this setting. In addition, tissue eosinophilia is not commonly linked with ALI.

Methods: Immunohistochemistry using a novel monoclonal antibody recognizing eosinophil peroxidase (EPX-mAb) was used to assess intrapulmonary eosinophil accumulation/degranulation. Lung biopsies from ALI patients (n=20) were identified following review of a pathology database; 45% of which (i.e., 9/20) displayed classical diffuse alveolar damage (ALI-DAD). Controls were obtained from uninvolved tissue in patients undergoing lobectomy for lung cancer (n=10). Serial biopsy sections were stained with hematoxylin and eosin (H&E) and subjected to EPX-mAb immunohistochemistry.

Results: EPX-mAb immunohistochemistry provided a >40-fold increased sensitivity to detect eosinophils in the lung relative to H&E stained sections. This increased sensitivity led to the identification of higher numbers of eosinophils in ALI patients compared with controls; differences using H&E staining alone were not significant. Clinical assessments showed that lung infiltrating eosinophil numbers were higher in ALI patients that survived hospitalization compared with non-survivors. A similar conclusion was reached quantifying eosinophil degranulation in each biopsy.

Conclusion: The enhanced sensitivity of EPX-mAb immunohistochemistry uniquely identified eosinophil accumulation/degranulation in patients with ALI relative to controls. More importantly, this method was a prognostic indicator of patient survival. These observations suggest that EPX-mAb immunohistochemistry may represent a diagnostic biomarker identifying a subset of ALI patients with improved clinical outcomes.

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POSTER C-11
ROLE OF TRAIL IN RHINOVIRUS INFECTION AND EXACERBATION OF ASTHMA
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Background: Rhinovirus is the most commonly detected pathogen in the airways of exacerbated asthmatics, the inflammation from which persists long after the virus has been cleared. TRAIL has already been shown to be a crucial molecule in the pathogenesis of allergic airways disease [1], and more recently in the modulation of immune cells vital to anti-viral responses.

Objectives: To elucidate the role of TRAIL at the point of rhinovirus infection that lead to the exacerbation of pre-existing allergic airways disease in vivo.

Methods: Naïve and allergic House Dust Mite (HDM) challenged wildtype and TRAIL-deficient Balb/c mice were intranasally infected with 1x10⁷ TCID50 of human minor serotype RV1B. Airways hyperresponsiveness (AHR) was invasively assessed 24 hours following infection by total airways resistance in response to increasing methacholine challenge (Buxco). Additionally, inflammation was assessed through enumeration of BAL cell populations, chemokine release was quantified by employing a fluorescent-bead multiplex platform on whole lung homogenates, and viral titre by quantitative RT-PCR and TCID50.

Results: TRAIL-deficient mice were protected from RV-induced AHR and had suppressed cellular infiltration into the airways in both the non-allergic and allergic environment. Chemokine levels were broadly suppressed in the TRAIL-deficient mice, and less RV1B was detected 24 hours following infection in naïve mice whereas no difference was detected in those challenged with HDM.

Conclusions: TRAIL is an important proinflammatory factor in primary rhinovirus infection in both a non-allergic and allergic lung environment, implicating this molecule as a possible target for the prevention of virally-induced asthma exacerbations.


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RESLIZUMAB IN THE TREATMENT OF POORLY CONTROLLED ASTHMA IN PATIENTS WITH EOSINOPHILIC AIRWAY INFLAMMATION

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Background: Eosinophilic asthma is a subtype of severe asthma characterized by persistence of eosinophils in the lung and sputum. IL-5 is involved in the maturation, recruitment, and activation of eosinophils.

Objective: To evaluate the effect of the humanized antibody to IL-5, reslizumab, on asthma symptoms in patients with asthma and eosinophilic airway inflammation.

Methods: Patients with poorly controlled asthma on high dose inhaled corticosteroids and ≥3% sputum eosinophils were randomly assigned to receive infusions of reslizumab 3.0 mg/kg or placebo at weeks 0, 4, 8, and 12 with stratification by baseline Asthma Control Questionnaire (ACQ) score ≤2 or >2. The study had IRB approval, and patients provided informed consent. The primary endpoint was change from baseline to week 15 in ACQ score. Secondary endpoints were measures of lung function and number of clinical asthma exacerbations (CAEs). A CAE was defined as a ≥20% decrease from baseline in FEV1, emergency treatment or hospitalization for asthma, or oral corticosteroids for ≥3 days.

Results: 106 subjects (mean age 45.4 years), poorly controlled (mean ACQ 2.7), eosinophilic-predominant asthma (median sputum eosinophils 10%) were enrolled. 8% had aspirin sensitivity, 14% had atopic dermatitis, 29% had chronic sinusitis, and 80% had allergic rhinitis. Overall, there was a trend toward improvement in asthma control associated with a significant improvement in lung function and a decrease in airway eosinophilia (Table). 8% of patients in the reslizumab group and 19% in the placebo group had a CAE (p=0.0833). Significantly greater improvements in ACQ score were observed with reslizumab versus placebo in patients with nasal polyps. The most common adverse events (AEs) with reslizumab (≥3 patients) were nasopharyngitis, fatigue, and pharyngolaryngeal pain. The AE profile of reslizumab and placebo were similar and no drug-related serious AEs were observed.

Conclusion: In patients with severe, poorly controlled eosinophilic asthma, reslizumab improved lung function and airway eosinophilia with a trend towards improved asthma control that was not statistically significant. In a subgroup with nasal polyps, reslizumab demonstrated an even greater improvement in asthma control. Reslizumab was generally well tolerated.

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AGING DECREASES EOSINOPHIL AND TH2 AIRWAY INFLAMMATION IN THE TRIPLE ANTIGEN MURINE ALLERGIC ASTHMA MODEL

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BACKGROUND: As the U.S. population ages, the prevalence of asthma in the older adult population is expected to increase significantly. Whether there are unique features of airway inflammation in this asthma population is not well understood. In vivo animal models have been used to study the impact of age on asthma and have provided valuable information on asthma pathogenesis, including features of airway inflammation. The animal asthma model most widely used is the mouse ovalbumin model. Despite its wide use, it has been difficult to attain features of chronic asthma as mice develop tolerance when chronically exposed to the same allergen. Goplen et al recently showed that sensitization and challenge with multiple allergens breaks through tolerance and generates a chronic allergic asthma-like phenotype in mice [1].

OBJECTIVES: To evaluate age-related differences in the airway inflammatory response in this new triple antigen murine model of allergic asthma.

METHODS: Young (8 weeks) and older (8, 12 and 18 months) female C57/BL6 mice were sensitized and intranasally challenged with a combination of dust mite, ragweed, and Aspergillus species in alum for 2 weeks [1]. A control group was sensitized with dust mite, ragweed and Aspergillus but challenged with saline. Total inflammatory cell counts and differential counts were determined in bronchoalveolar fluid (BALF) and inflammatory cytokines were measured in the cell-free BALF by multiplex immunoassay.

RESULTS: In the mice that underwent both sensitization and challenge with the triple antigens, the BALF eosinophil numbers were significantly lower then the in the aged 12- and 18-month-old mice compared to the younger 8-week and 8-month-old mice. In addition to their decreased eosinophil response, the 12- and 18-month-old mice also had significantly lower BALF total cell numbers. Th2 cytokine responses (IL-4, IL-5) and eosinophil chemotactic chemokines (eotaxin, RANTES) were significantly reduced in the 12- and 18-month-old mice compared to the 8-week and 8-month-old mice.

CONCLUSIONS: The acute triple antigen asthma model produced a unique airway inflammatory cell (↓eosinophils) and cytokine/chemokine profile (↓IL-4, IL-5, eotaxin, RANTES) in the aged mice. These findings suggest that Th2 and eosinophilic response to acute antigen sensitization and challenge are reduced in older mice leading to a different phenotype (non-eosinophilic) of allergic asthma, which requires further characterization and similar assessments in the chronic triple antigen murine model.


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**POSTER C-14**

**INCREASED SENSORY INNERRATION AND VAGALLY-MEDIATED AIRWAY RESISTANCE IN A MOUSE MODEL OF EOSINOPHILIC ASTHMA**

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**Background:** Hyperresponsive sensory signaling contributes to airway hyperreactivity in asthma/COPD but the underlying mechanism(s) remains understudied. In other organs (e.g. skin, bowel), sensory hyperresponsiveness is associated with inflammation and branched outgrowth of peripheral sensory nerves. We had previously shown that eosinophils, an inflammatory cell associated with asthma, increase dorsal root ganglion sensory neuron branching in vitro. However, in lung tissue quantifying nerve branching and growth in airway tissue has been hindered by the complex three-dimensional structure of airway sensory nerves.

**Objectives:** We hypothesize that eosinophilic airway inflammation increases branched outgrowth of airway sensory neurons and increases reflex bronchoconstriction.

**Methods:** We overcame the hurdle of quantifying structural changes to airway sensory nerves by developing a computer modeling analysis of three-dimensional nerve images. We then used this method to quantify nerve length/branching in wildtype mice and transgenic mice with overabundant airway eosinophils (IL5 driven by the airway epithelial specific CC10 promoter). Using our new computer modeling analysis, we now show that epithelial sensory nerves in eosinophil transgenic mice contain double both the number of branchpoints (83.8 ± 7.0 vs 39.6 ± 10.4) and total neurite length (2126.4μM ± 415.4 vs. 1084.3μM ± 166.2). Preliminary airway resistance experiments show eosinophil transgenic mice exhibit augmented reflex responses to aerosolized serotonin, an effect inhibited by vagotomy.

**Conclusions:** We conclude that eosinophils promote dramatic airway sensory nerve growth, and that this may potentiate reflex bronchoconstriction.

**Grant Support:** This work was supported by NIH T32AI007472 (DBJ) and the Mayo Foundation for Medical Education and Research as well as NIH HL61013 (DBJ), HL71795 (DBJ), AI75064 (DBJ), HL55543 (ADF), ES14601 (ADF), HL065228 (J JL), RR109709 (J JL), and HL058723 (NAL).
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POSTER D-1

EOSINOPHIL CONTROL OF THE NEMATODE STRONGYLOIDES STERCORALIS IN THE INNATE IMMUNE RESPONSE: MOLECULAR BASIS OF EOSINOPHIL CHEMOTAXIS AND PARASITE KILLING

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Background: Eosinophils can function in protective immunity to the nematode Strongyloides stercoralis as both effector cells in innate immunity and as antigen presenting cells.

Objectives: The goals of this study were to determine: (1) how eosinophils are recruited to the nematode S. stercoralis, (2) whether eosinophils were required for innate and adaptive immunity and (3) the molecular mechanism through which eosinophils kill the parasite.

Methods and Results: Eosinophils undergo both chemotaxis and chemokinesis to soluble parasite extract in transwell plates. CCR3, CXCR4 or CXCR2 antagonists significantly inhibited eosinophil chemotaxis to the parasite extract. Treating the extract with proteinase K or chitinase significantly inhibited its ability to induce chemotaxis, thereby demonstrating that the chemoattractants were both protein and chitin. Therefore, chemoattractants derived from parasites and host species stimulate similar receptors to induce eosinophil chemotaxis. Protective innate immunity to S. stercoralis requires eosinophils in the killing process based on increased parasite survival after treatment with anti-CCR3 antibody. Infecting PHIL mice, which are eosinophil deficient, with S. stercoralis resulted in development of innate and adaptive immune responses that were similar to wild type mice suggesting that eosinophils are not an absolute requirement for larval killing or development of secondary immunity. Treating PHIL mice with a neutrophil-depleting antibody resulted in a significant impairment in larval killing. Using mice deficient in the eosinophil granule products major basic protein (MBP) and eosinophil peroxidase (EPO), it was determined that if neutrophils were absent, the innate immune response required eosinophils to kill the larvae through an MBP dependent mechanism.

Conclusion: Although eosinophils are not required for innate or adaptive immunity to S. stercoralis it is clear that they are attracted to parasite molecules and that they are capable of killing the worms though a MBP dependent mechanism.

References:

Grant Support: This work was supported in part by NIH grants RO1 AI47189 (DA), 1R56 AI076345 (DA). NCRR K26 RR0109709 (JJL) and Mayo Foundation for Medical Foundation for Medical Education and Research (JJL).
Background: Parasite-derived lipids may play important roles in host-pathogen interactions and escape mechanisms. Remarkable accumulation of eosinophils is a characteristic feature of inflammation associated with parasitic disease, especially caused by helminthes. Infiltrating eosinophils are strongly implicated in the pathogenesis of these disorders by virtue of their capacity to release an array of tissue-damaging and immunoregulatory mediators. However, the mechanisms involved in the direct activation of eosinophils by parasite-derived molecules are not clear.

Objectives: We investigated whether eosinophils can be directly activated by schistosomal lipids. Further we evaluated the mechanisms involved in schistosomal lipids-triggered eosinophil activation focusing on putative roles of eosinophil-expressed Toll-like 2 receptor (TLR2) and schistosomal prostaglandin 2 (PGD2).

Methods: Purified human eosinophils were incubated for 1 h at 37°C with schistosomal lipid extract or schistosomal-derived lysocephatidylcholine (Lyso-PC), arachidonic acid or PGD2. Pre-treatments were performed for 30 min with: neutralizing antibody against TLR2 or PGD2 receptor antagonists (BWA868 for DP1 and Cay10471 for DP2 receptors). TLR2 mRNA and protein levels were analyzed by RT-PCR and Western Blotting, respectively. Lipid body formation was studied by Nile Red staining and ADRP expression. Quantification of cytokines, LTC4 and EXC4 (14,15 LTC4) levels was carried out by ELISA, LUMINEX and EIA kits. Immunodetection of intracellular EXC4 was determined by EicosaCell assay.

Results: The extract of total schistosomal lipids as well as isolated schistosomal Lyso-PC are capable of activating human eosinophils, eliciting lipid body biogenesis and increased ADRP expression, synthesis of LTC4 and EXC4 and differential secretion of pre-formed cytokines. TLR2 is expressed in basal levels in human eosinophils and it is up-regulated upon stimulation with schistosomal lipids. Pre-treatment with anti-TLR2 inhibited both schistosomal lipids- and Lyso-PC-induced lipid body biogenesis within eosinophils, indicating that TLR2 mediates schistosomal lipids- and Lyso-PC-driven eosinophil activation and is expressed on eosinophils. On the other hand, by employing PGD2 receptor antagonists, we unveiled that DP1 and DP2 receptors are also involved in various parameters of eosinophil activation (such as lipid body biogenesis, synthesis of LTC4 and EXC4 and cytokine secretion) induced by schistosomal lipids, but not by schistosomal Lyso-PC. In agreement, we also showed that, besides Lyso-PC, PGD2 is another lipid component of schistosomal lipids.

Conclusions: Taken together, our results showed that schistosomal lipids contain at least two components that are capable of direct activation of human eosinophils. Both schistosomal Lyso-PC and PGD2 acting on distinct eosinophil-expressed receptors, noticeably TLR2 as well as DP1 and DP2, differentially trigger eosinophil activation characterized by production/secrection of pro-inflammatory and immunoregulatory mediators.

Grant Support: This work is funded by CAPES, CNPq, FAPERJ and NIH.
POSTER D-3

EOSINOPHIL-DERIVED IL-6 IS REQUIRED FOR PARASITE CLEARANCE DURING INFECTION WITH LITOMOSOIDES SIGMONTIS

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Background: Lymphatic filariasis is caused by the filarial nematodes Brugia malayi and Wuchereria bancrofti. Symptoms include lymphoedema and hydrocele formation. Eosinophilia is associated with filarial infection though the role of eosinophils remains to be elucidated.

Objectives: This study investigated the role of eosinophils during filarial infection using several gene-deficient mice to characterize immune responses in a mouse model of filarial infection.

Methods: Balb/c, Eotaxin-1-/-, IL-6-/- and ΔdblGATA mice were infected with the murine filarial nematode Litomosoides sigmodontis. Mice were sacrificed at different time points post infection (corresponding to the life stages of the worms) and worm burden in the pleural cavity as well as local and systemic immune responses were determined. We measured local cytokine production in the pleural cavity by ELISA and determined the cellular composition of pleural exudate cells by FACS for the local immune responses. Systemically, we investigated the generation of specific antibodies and the splenic recall response.

Eosinophils were then purified using magnetic bead sorting and stimulated in vitro with soluble extracts from L. sigmodontis (LsAg). Activation was determined by ELISA (IL-6, MIP-2 production) and FACS analysis (MHCII, CD62L expression). IL-6 production ex vivo was determined by intracellular flow cytometry.

Results: Eotaxin-1-/- mice show increased worm burden during chronic infection (60 dpi) with a normal systemic (IgG level, splenic IL-5 and IFNγ production) response. Locally, normal cellular infiltration into the pleural cavity was observed. Specifically, eosinophil migration was not altered. We reasoned that increased worm burden might therefore be due to decreased eosinophil function and stimulated eosinophils from Balb/c and Eotaxin-1-/- mice with LsAg. We found decreased production of IL-6 by Eotaxin-1-/- eosinophils. This finding was confirmed in vivo where decreased levels of IL-6 were found in the pleural cavity of infected Eotaxin-1-/- mice. Intracellular staining of IL-6 in pleural cavity eosinophils further confirmed IL-6 production by eosinophils. To further confirm this finding, we infected eosinophil deficient ΔdblGATA mice with L. sigmodontis. We found increased worm burden but no eosinophils in the pleural cavity of ΔdblGATA mice. There were also decreased levels of IL-6 in the pleural cavity. Finally, we infected IL-6-/- mice with L. sigmodontis. As expected IL-6-/- mice showed an increased worm burden in the pleural cavity as early as 15 dpi although eosinophil migration to the pleural cavity was even increased in IL-6-/- mice.

Conclusion: With this study, we demonstrated a role for Eotaxin-1 in parasite survival during filarial infection. This was not due, however, to decreased eosinophil migration but to decreased eosinophil activation. This was confirmed by experiments in vivo and in vitro where Eotaxin-1-/- eosinophils produced decreased levels of IL-6. Further confirmation was obtained from eosinophil deficient mice that also showed reduced levels of IL-6 in the pleural cavity. Finally, even though eosinophil infiltration into the pleural cavity was increased in IL-6-/- mice, these eosinophils were not able to clear filarial infection also resulting in a higher worm burden in these mice. We could here demonstrate using several gene-deficient mice that eosinophil-produced IL-6 is required for parasite clearance during filarial infection.
A COMPARATIVE ANALYSIS OF INFLAMMATORY REACTIONS IN LOA LOA AND ONCHCERCA VOLVULUS INFECTED PATIENTS AFTER TREATMENT WITH DIETHYLCARBAMAZINE

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Background: Diethylcarbamazine citrate (DEC) treatment of loiasis is complicated by the development of severe adverse reactions that are correlated with the number of circulating microfilariae in the blood. The cause of post-treatment reactions in filariasis is unknown, but they are accompanied by a dramatic interleukin-5 (IL-5)-dependent increase in eosinophilia and evidence of eosinophil activation. Although release of the intracellular bacterial endosymbiont, Wolbachia, is thought to contribute to post-treatment reactions in some filarial infections, including onchocerciasis, Wolbachia is not present in Loa loa parasites.

Objectives: We hypothesize that the immunological profile following DEC treatment will have a Th2 predominance in subjects with loiasis; whereas subjects with onchocerciasis will have a mixed Th1/Th2 picture.

Methods: The immune profiles after DEC treatment of subjects with microfilaria-positive loiasis and onchocerciasis were compared using data and stored serum samples from two prior clinical trials. Samples were collected at baseline and at 1, 2, 3, 4, 5, 6, 7, and 14 days post-treatment from 2 Loa loa infected subjects treated with DEC (8-10 mg/kg for 21 days) at the NIH and 6 O. volvulus infected subjects treated with DEC (200 mg daily for 7 days) in Ghana. Post-treatment eosinophil and neutrophil counts were compared. Serum levels of soluble IL-5 receptor-α (sIL-5Rα) were quantified by ELISA. Suspension array technology in multiplex was used to assess serum levels of IL-1β, IL-2, IL-5, IL-8, IL-10, IL-13, TNF-α, IFN-γ, MIP-1β, eotaxin, GM-CSF, and G-CSF.

Results: The absolute eosinophil count (AEC) rose post-DEC treatment in all 8 subjects, peaking at 4-6 days in the 2 subjects with loiasis, but continuing to rise through the 14 days post- treatment in all 6 subjects with onchocerciasis. The absolute neutrophil count (ANC) also rose post-treatment and showed a bimodal pattern peaking at day 1-3 and then again at day 6 in both subjects with loiasis and 5/6 subjects with onchocerciasis. A rise in the serum IL-5 level preceded the increase in AEC by 1-2 days in all subjects (median increase from 0-62.3 pg/ml, p=0.008, Wilcoxon signed rank test), and serum sIL-5Rα levels increased in parallel with the AEC. Serum IL-10 and TNF-α levels increased significantly in all subjects at 1 day post-treatment (p=0.008 and 0.015 respectively). Interestingly, serum IL-8 levels increased compared to baseline levels on post-treatment day 1-2 in the 2 subjects with loiasis, but decreased in 5/6 subjects with onchocerciasis. A similar, but less consistent, pattern was seen for serum MIP-1β levels. Serum levels of the other cytokines measured were either undetectable or stable post-treatment.

Conclusions: Although post-treatment reactions in both loiasis and onchocerciasis are associated with IL-5-mediated eosinophilia, the kinetics of the responses are consistent with a more rapid effect of DEC on parasite clearance in loiasis. Despite the absence of Wolbachia in Loa loa parasites, no post-treatment differences were seen in the neutrophil responses between subjects with loiasis and those with onchocerciasis. Assessment of neutrophil activation markers, including myeloperoxidase, lactoferrin, and MMP-9 is currently underway.

Support: This study was supported by the Division of Intramural Research, NIAID, NIH. Clinical trial NCT00001230.
POSTER D-5

ECP (EOSINOPHIL CATIONIC PROTEIN) GENETICS IN VISCERAL LEISHMANIASIS

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Background: Visceral Leishmaniasis (VL) also known as kala-azar is caused by the protozoa of Leishmania Donovani which is introduced into the human host by the bite of a female sandfly [1]. The parasite rapidly invades macrophages, where they multiply inside phagolysosomes. Fifty % of subjects treated for VL develop the serious skin lesion post-kala-azar dermal leishmaniasis (PKDL).

Objectives: To investigate the possible role of the eosinophil and specifically the role of one of the major secretory proteins, ECP, in VL and PKDL.

Methods: In total 282 subjects were investigated of which 174 had VL and 108 subjects from the same area served as controls. Of the 174 subjects with VL 75 had developed post treatment PKDL. Blood was drawn for the purpose of ECP genotyping and for the measurements of serum and cellular levels of ECP. Sample sequencing of the ECP gene was performed on Beckman Coulter CEQTM 2000 and 8000 DNA analysis systems, and/or by the TaqMan 5’ nuclelease allelic discrimination assay. ECP was measured by a sensitive and specific ELISA.

Results: Five ECP gene polymorphisms; ECP113(T>C) [2], ECP c.-38(A>C) rs2233859, ECP434(G>C) rs2073342, ECP562(G>C) rs2233860 and ECP1088(A>T) rs8019343 were identified by gene sequencing. In two subjects an ECP gene deletion was identified. Genotype prevalences of ECP c.-38(A>C) and ECP434(G>C) showed ethnic differences in comparison to a Swedish cohort (p<0.0001). ECP113(T>C) and ECP1088(A>T) SNPs were unique to the Sudanese population. The ECP c.-38 C-allele was more frequent in the VL cohort as compared to the healthy control group (p=0.001). Those affected by PKDL had a higher prevalence of the C-allele as compared to the VL only cohort (p=0.005). The other genotypes did not show any differences. The combination of ECP genotypes did not add any further to the findings of the ECP c.-38(A>C) associations.

The eosinophil content of ECP was significantly reduced in subjects with VL as compared to controls (p=0.0002), but with no differences between subjects with VL only or PKDL. Nor did we find any relations between the eosinophil content and any of the ECP genotypes. In the two subjects with ECP gene deletion the eosinophils lacked ECP.

Conclusions: Our findings show major differences between Scandinavian and Sudanese populations as to the prevalences of several ECP gene polymorphisms. We conclude that ECP may play a role in the susceptibility to infections by Leishmania Donovani and the development of the serious skin lesion post-kala-azar dermal Leishmaniasis.


Grant Support: This project was funded by The Swedish Medical Research Council, The Swedish Heart and Lung Foundation, The Asthma and Allergy Research Foundation, Bror Hjerpstedts and Agnes and Mac Rudbergs Foundations.
# E — POSTER SESSION ABSTRACTS

## EOSINOPHILIC GASTROINTESTINAL DISEASE (EGID)

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MALE SEX OF AFFECTED PARENT IS ASSOCIATED WITH FAMILIAL EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) occurs more frequently in males and in families with allergies and atopy.

Objectives: We will quantify the risk of developing EoE and related conditions in first degree relatives of affected individuals, stratified by sex.

Methods: A retrospective, cross sectional study was conducted for the period August 2008 to July 2010 to identify proband patients with documented family history. Previsit parent questionnaire with MD confirmation was conducted for family relations and their comorbid conditions, i.e., allergic rhinitis, asthma, eczema, food allergies, urticaria, EoE, other eosinophilic gastrointestinal disorders (EGID), food impaction, esophageal dilation. Pedigrees were constructed, using PEDSYS and SOLAR software, from a database with first degree relation information for 29%. Recurrence risk ratios (RRR) were calculated as (#affected/total)/prevalence. Data were analyzed with Chi-square and Fisher’s Exact at p<=0.05.

Results: This sample had 1.77 siblings per family compared to the Ohio mean of 1.87 and the US mean of 1.86. Pedigrees were constructed for 306 families of proband patients. First degree relatives affected with EoE included 3.3% of fathers, 0.4% of mothers, 2.4% of brothers and 2.9% of siblings overall. Fathers are affected significantly more frequently than mothers (p=0.03). The RRR is 33 and 54, for parents and siblings, 62 and 21 for males and females, and 61, 8, 64, 44 for fathers, mothers, brothers, sisters, respectively. All are significantly increased compared to population prevalence. EGID (p=0.017), food impaction (p=0.001) and esophageal dilation (p=0.002) were significantly more common in parents compared to siblings. EoE (p= 0.024) and food impaction (p=0.039) were reported more frequently by fathers than mothers. Asthma (p=0.001) and eczema (p=0.0001) were more common in siblings of probands compared to parents.

Conclusions: In conclusion, we have defined specific risk ratios of EoE in first degree relatives with a range of 8-64 depending upon relationship and sex; fathers were more commonly affected with EoE compared with mothers. Parents and siblings show distinctly different patterns of comorbid conditions. However, recurrence risk does not differentiate between genetic and environmental contributions to disease. Further studies of sex-based inheritance patterns and family-based quantification of shared environment are warranted.

Grant support: This work is funded by the Buckeye Foundation, CURED, Food Allergy Initiative, NIAID, NIDDK, NIHT32ES10957, DODW81XWH-10-1-0167.
CDH26 EXPRESSION AND FUNCTION IN EOSINOPHILIC GASTROINTESTINAL DISORDERS

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Background: Eosinophilic gastrointestinal disorders (EGID) encompass a spectrum of diseases defined by abnormal accumulation of eosinophils in particular segments of the GI tract. Eosinophilic gastritis (EG) and eosinophilic esophagitis (EE) represent diseases characterized by accumulation of eosinophils in the stomach or esophagus, respectively.

Objectives: The aims of this study include defining a molecular signature in gastric tissue of patients with EG and understanding the molecular pathways that underly the pathogenesis of this disease. We focus on understanding the expression pattern and function of cadherin-like 26 (CDH26) in EG and EE, as this is a gene product markedly overexpressed in EGID tissue.

Methods: Global transcript analysis was performed to identify genes differentially expressed in the gastric tissue of patients with active EG compared to control individuals. Further characterization of the gene and protein expression patterns of CDH26 was undertaken through real-time PCR, immunohistochemistry, and western blot analysis. CDH26 protein interactions were examined using transient transfection and immunoprecipitation analysis.

Results: A conserved set of 28 genes were upregulated and 76 were downregulated in gastric tissue of patients with active EG compared to control patients. Of these genes, 11 overlapped with those previously identified as being dysregulated in esophageal tissue of patients with EE including CDH26, which represented the most highly overexpressed gene in EG biopsies (20.9-fold, p<0.01). We observed that epithelial cells exhibited increased CDH26 protein expression in both esophageal and gastric tissue of patients with active EE or EG, respectively. Similar to EE, IL-13 transcript levels were highly increased in the gastric tissue of patients with active EG (375-fold, p<0.01). IL-13 induced CDH26 expression in primary esophageal epithelial cells, TE-7 esophageal epithelial cells, and NCI-N87 gastric cells in vitro. CDH26, an uncharacterized member of the cadherin superfamily of proteins, exhibited homotypic interaction and additionally interacted with beta-catenin, alpha-catenin, and p120/delta-catenin when expressed ectopically in 293T cells.

Conclusions: We gained insight into the molecular pathways involved in EG pathogenesis by identifying a signature of genes commonly dysregulated in the gastric tissue of EG patients. Our data further suggest that CDH26 may be regulated in part by IL-13 in esophageal and gastric epithelial cells. CDH26 interacts with cadherin proteins, which link cadherin molecules to the actin cytoskeleton. Furthermore, CDH26 exhibits homotypic interaction, consistent with a function of CDH26 in cell adhesion. As such, we propose that CDH26 is a major cadherin expressed in allergic GI tissue and as such may have a key role in various aspects of disease pathogenesis and diagnosis.

Grant Support: This work is funded by American Heart Association Fellowship (J.M.C), NIH grants R01 DK76893 and U19 AI070235 (M.E.R.), the Food Allergy Project (M.E.R.), the Buckeye Project, and the CURED (Campaign Urging Research for Eosinophilic Disease) Foundation.
COMPARATIVE PROTEOMIC ANALYSIS IN EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) is an inflammatory disease characterized by eosinophil infiltration of the mucosal lining of the esophagus. Clinical symptoms do not correlate with mucosal disease and are often indistinguishable from gastroesophageal reflux disease (GERD). There is no serologic marker for disease, but peripheral blood eosinophils may show evidence of changes in protein expression useful in differentiation of EoE from GERD and normal.

Objectives: We hypothesize that the proteome pattern detected in peripheral blood eosinophils will differ between EoE, GERD and normal subjects.

Methods: Leukocytes were isolated from venous blood drawn at the time of endoscopy from donors with EoE and GERD symptoms by density centrifugation with HISTOPAQUE-1077. Subjects were classified into EoE, GERD and normal groups based on pathologic diagnosis. Eosinophils were isolated by negative selection using CD16 MicroBeads. 2-D gel electrophoresis was performed with isoelectric focusing by a multi-sample IPGphor (Zoom® Fractionator) followed by fluorescent staining with Pro-Q Diamond and Sypro Ruby. Stained gels were imaged at 100 μm resolution and analyzed using Progenesis SameSpots software v3.3. Selected spots were robotically picked, trypsin digested, and then transferred to MALDI-MS target plates. MALDI TOF/MS were used to analyze tryptic peptide samples and determine protein identification. Data was acquired with an Applied Biosystems 4800 MALDI-TOF/TOF Proteomics Analyzer. GPS Explorer TM (v3.6) software was used with MASCOT to search protein databases for protein identification. Protein match probabilities were determined using expectation values and/or Mascot protein scores. Protein identification was determined through the NCBI or SwissProt databases. Expectation values of <0.01 were considered significant.

Results: Twenty nine proteins were found to have at least a 2-fold difference in expression between the EoE, GERD and normal subjects. Seventeen proteins were upregulated in EoE compared to normal and 20 proteins were upregulated in EoE compared to GERD. Fourteen and eight proteins were downregulated in EoE when compared to GERD and normal, respectively. We identified eleven proteins which had Mascot expectation scores of 10(-3) or less, with at least 8 of these proteins upregulated three fold in EoE compared to GERD. Significant changes were noted in several proteins involved in inflammatory responses, cell adhesion and apoptosis. Evidence of post-translational modification was found in eleven proteins with the majority of these proteins involved in chemotaxis.

Conclusions: We have demonstrated differences via comparative proteomic analysis in EoE, GERD and normal subjects with significant upregulation of expression of specific proteins in peripheral blood eosinophils of EoE patients. Changes in peripheral eosinophil protein expression were identified between EoE, GERD and normal subjects. The proteins identified here will be important in determining potential new biomarkers of disease in EoE.


Grant Support: This work is funded by Texas Children’s Hospital Pediatric Pilot Fund Grant Award, NIH NHLBI Proteomics Initiative NO1-HV-00245.
IDENTIFICATION OF ESOPHAGEAL MICROBIOTA IN EOSINOPHILIC ESOPHAGITIS AND GASTROESOPHAGEAL REFLUX DISEASE

Sophie Fillon¹, Caleb J. Kelly¹, Shauna Schroeder¹, Wendy Moore¹, J. Kirk Harris³, Brandie D. Wagner², Mark Stevens², Charles E. Robertson², Joanne C. Masterson¹, Steve Ackerman⁴ and Glenn T. Furuta¹

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Background: Esophagitis is characterized by upper intestinal symptoms found in association with chronic inflammation. The two most common forms of esophagitis are related to acid injury, gastroesophageal reflux disease (GERD) and allergy. Eosinophilic Esophagitis (EoE). To date, the underlying etiology of these diseases is not certain but an emerging body of data implicates microbial triggering underlying some forms of gastrointestinal inflammation. Few studies have determined the microbiome of the esophagus in health or disease and those have utilized mucosal biopsies. The Enterotest utilizes a swallowed nylon string in a capture device to detect intestinal parasites.

Objectives: The aim of this study was to identify the esophageal microbiome in well-defined patients with esophagitis. We hypothesize that the microbiome varies depending on the state of esophageal inflammation and may act as an inciting factor.

Methods: Nose swab, oral, esophageal string samples and mucosal biopsies were collected from children with EoE (n=11) and GERD (n=5) as well as normal controls (n=14). All patients had upper intestinal symptoms with further diagnostic criteria including EoE- > 15 eos / high power of field (HPF) and other causes excluded; GERD- < 15 eos / HPF and/or abnormal pH/impedance study; normal controls- normal esophageal mucosa. Microbiome was determined by 16S rDNA amplification and 454 pyrosequencing. Sequencing results were analyzed by subject group.

Results: Variations existed between microbiome measured in the esophagus compared to that identified in the mouth and nose. No significant differences in bacterial genera were observed between the microbiota on biopsies and string in healthy and inflamed patients. During various disease states, differences in esophageal microbiota were observed as shown in the Table below.

<table>
<thead>
<tr>
<th>Bacterial Genus</th>
<th>Normal</th>
<th>EoE</th>
<th>GERD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>25.8</td>
<td>23.9</td>
<td>16.1</td>
</tr>
<tr>
<td>Pasteurella</td>
<td>4.8</td>
<td>2.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Haemophilus</td>
<td>2.5</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Hallella</td>
<td>1.2</td>
<td>2.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Weeksella</td>
<td>2.0</td>
<td>8.1</td>
<td>6.4</td>
</tr>
</tbody>
</table>

(Numbers represent the median of the % sequences in patients with esophagitis or not.)

Conclusions: Esophageal inflammation in children is associated with esophageal microbiome alterations. Streptococcus was decreased on the esophageal string from children with GERD. Minimally invasive string technology can detect microbiome of the esophagus and is comparable to mucosal biopsy.

Grant Support: This work was funded by 2010 American Partnership For Eosinophilic Disorders Junior Faculty HOPE Research Grant and KL2 grant from the Colorado CTSI. The abstract was supported by NIH/NCCR Colorado CTSI Grant Number UL1 RR025780. Its contents are the authors’ sole responsibility and do not necessarily represent official NIH views.
Poster E-5

EOSINOPHILS MODULATE THE COMPOSITION OF STOOL MICROBIOTA

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Gastrointestinal Eosinophilic Diseases Program and Mucosal Inflammation Program, University of Colorado Denver School of Medicine Aurora, CO, Department of Pediatrics, National Jewish Health Denver CO, Digestive Health Institute and Section of Pediatric Gastroenterology, Hepatology and Nutrition, The Children’s Hospital, Aurora CO 1 Division of Pulmonary Medicine, The Children’s Hospital, Aurora, CO, University of Colorado Denver, School of Medicine, 2 Mayo Clinic Scottsdale, S.C. Johnson Medical Research Center, Scottsdale, Arizona. 3

Background: Alterations in the composition of colonic microbiota with depletion of commensal bacteria including Firmicutes and Bacteroidetes are increasingly recognized as a key factor in the pathogenesis of IBD. Eosinophils generate a number of different molecules with anti-microbial properties and the juxtaposition of intestinal eosinophils to the epithelium with its bacteria laden luminal surface suggests they may participate in the maintenance of intestinal immune homeostasis.

Objectives: The aim of this study was to determine the effect of eosinophils on the composition of the gastrointestinal microbiota. We hypothesize that resident intestinal microbiota populations are modulated by eosinophils.

Methods: To address this hypothesis, we studied wild type (WT) C57/BL6 and eosinophil deficient (PHIL) littermates. Mice were housed in the same animal facility under specific pathogen free (SPF) conditions and were fed the same chow. Between 8-12 weeks of age, C57/BL6 (n=12) and PHIL (n=9) female mice were sacrificed and stool pellets collected, and colonic and ileal tissues specimens were harvested for DNA isolation (Qiagen). Compositions of luminal (stool) and adherent (colon and ileum) microbiota was determined by metagenomic analysis of PCR amplified bacterial 16S rDNA using Denaturing Gradient Gel Electrophoresis (DGGE), 454 pyrosequencing, and phylogenic analysis.

Results: Measurement of microbial load by 16S quantification did not reveal differences between WT and PHIL mice. DGGE analysis identified differing band patterns comparing stool microbiome present in PHIL to WT mice and tissues. Pyrosequencing data revealed that the bacterial genus Shigella and Enterobacter were uniquely present in the stool microbiota in PHIL mice but not in WT mice.

Conclusions: Absence of eosinophils may increase the susceptibility of mice to colonization by opportunistic pathogens based on the presence of Shigella and Enterobacter in the PHIL mice. These results suggest that eosinophils play a role in maintaining homeostatic composition of the intestinal microbiota.

Grant Support: This work was supported by a Crohn’s Colitis Foundation of America Student Research Award and North American Society of Pediatric Gastroenterology, Hepatology and Nutrition Summer Student Award (CJ Kelly).
**POSTER E-6**

**DIVERGENT RELATIONSHIP BETWEEN CCL11/EOTAXIN-1 AND CCL24/EOTAXIN-2 AND EOSINOPHILS IN ACTIVE VS QUIESCENT ULCERATIVE COLITIS.**

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**Background:** Previous investigations have demonstrated a link between the eosinophil-selective chemokines; eotaxins (eotaxin-1/CCL11 and eotaxin-2/CCL24), IL-5, eosinophils, and the inflammatory bowel diseases Crohn’s disease and ulcerative colitis (UC). However the relative contribution of IL-5, CCL11 and CCL24 to eosinophil recruitment in active UC and quiescent UC and the cellular source of eotaxins in UC remain unclear.

**Objective:** The objective of this study was to define the relationship between CCL11, CCL24, IL-5 and eosinophil levels in active UC and quiescent UC and normal control subjects and to identify the cellular source of CCL11 in the intestine.

**Methods:** Rectal biopsy samples were obtained from 12 patients with active UC, 9 with quiescent UC and 7 control subjects. Expression levels of IL-5, CCL11 and CCL24 in biopsy samples were determined by quantitative PCR analysis. Macrophages / monocytes, and eosinophil levels and activation (CD69 expression) in rectum biopsy samples were analyzed by flow cytometry and the cellular source of CCL11 was visualized by immunofluorescence analysis.

**Results:** Intestinal eosinophil (CCR3+ CD15+) and CCL11 levels were elevated in rectum biopsy samples from both active and quiescent UC compared with control subjects (p < 0.001). Importantly, levels of CCL11 mRNA positively correlated with rectum eosinophil numbers, all patients included (p < 0.001). IL-5 and CCL24 mRNA levels were elevated in active UC (p < 0.014); however we observed no significant difference between quiescent UC and normal control subjects. Flow cytometry analyses revealed a significant increase in CD14+ CD33+ myeloid cells in active UC compared with control subjects (p < 0.05), and the levels of CD14+ CD33+ myeloid cells positively correlated with eosinophil numbers in active UC (p < 0.0048). The level of eosinophil activation (increased CD69 expression) was increased in active but not in quiescent UC. Immunofluorescence analysis revealed the presence of CCL11-positive CD33+ CD14+ mononuclear cells within rectum biopsy samples of active UC patients.

**Conclusions:** These data suggest 1) that CD33+ CD14+ intestinal inflammatory myeloid cells may be an important source of CCL11 and regulate eosinophil recruitment in both active and quiescent UC and 2) that CCL24 and IL-5 expression can distinguish between active UC and quiescent UC and may be involved in the activation of eosinophils. These studies identify an important role for the macrophage:CCL11:eosinophil axis in the pathophysiology of UC.
POSTER E-7
ANALYSIS OF PHENOTYPES OF TISSUE T-CELLS AT VARIOUS LOCATIONS IN THE ESOPHAGUS OF PATIENTS WITH EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis is thought to be mediated at least in part by an allergic mechanism, and is associated with presence of high numbers of CD8+ and CD4+ lymphocytes. Although this disease is associated with high levels of IL-5 and responds well to corticosteroids, the antigen/allergen and the underlying immune mechanism remain to be identified.

Objectives: Our aim was to phenotype lymphocytes from the esophagus tissue of eosinophilic esophagitis patients and compare these cells with T-cells from tissue of the healthy esophagus.

Methods: Biopsies were obtained from the proximal, mid and distal part of the esophagus from patients with eosinophilic esophagitis (EoE) and from healthy squamous epithelium of controls. The diagnosis of eosinophilic esophagitis was made on the presence of 20 or more eosinophils per high power field. Tissue T-cells were expanded in vitro and the phenotypes of these cells were analyzed by flowcytometry using antibodies directed against T-helper cells (CD4), cytotoxic T-cells (CD8), the cytotoxic protein granzyme B, the intraepithelial integrin CD103 (αE), NKg2a and the integrin subunits α4(CD49d) and β1(CD29). Intracellular staining for IFN-γ and IL-4 was performed to analyze the presence of Th1 and Th2 cells, respectively.

Results: Differences are present between T-cells isolated from normal tissue and tissue from EoE patients. The comparison between cells from controls and the distal part of the esophagus of EoE patients showed that T-cells from EoE patients: (i) had a higher percentage of CD8+GranzymeB+ T-cells, (ii) had a low number of CD4+CD103+ cells, and (iii) had a higher expression of β1 on both CD4+ T-cells and CD8+T-cells. Interestingly, the expression of α4 on CD4+/T-cells isolated from the proximal parts of EoE patients was higher than on cells from the distal parts of the esophagus of EoE patients and of controls. No differences were found in α4 expression on CD8+ T-cells from various segments of EoE esophagus and control tissue. In two out of four patients, a large difference was observed in presence of CD3+CD4+ cells between proximal and distal segments of the esophagus: 71% (pt1) and 62% (pt2) CD3+CD4+ cells vs. 25%(pt1) and 35% CD3+CD4+ cells, in the proximal and distal parts of the esophagus, respectively. However, the significance of this finding remains to be established. This difference in CD3+CD4+ cells was not observed in the segments of the esophagus of controls. T-cells from both control tissue and EoE were characterized by an IFN-γ response upon in vitro activation (21±15% CD4+IFN-γ+ cells, 41±17% CD8+IFN-γ+ cells in EoE vs. 4±3%CD4+IFN-γ+ in and 58±36%CD8+IFN-γ+ in controls). No IL-4 producing cells were found.

Conclusions: The data of this pilot study are consistent with the hypothesis that the proximal segment of EoE is associated with an inflammatory CD4+α4β1+ response, whereas the distal segment of the esophagus of EoE is associated with cytotoxic CD8+GranzymeB+ response. No indications for the presence of Th2 cells were found.


Grant Support: This work is funded by Dutch Digestive Foundation, (nr. WO 06-26)
**POSTER E-8**

**EOSINOPHILS IN INTESTINAL INFLAMMATION & REMODELING**

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¹Section of Pediatric Gastroenterology, Hepatology and Nutrition, The Children’s Hospital Denver, ²Gastrointestinal Eosinophilic Diseases Program, ³Mucosal Inflammation Program, ⁴Division of Gastroenterology, ⁵Department of Pathology, ⁶University of Colorado Denver School of Medicine Aurora, CO, USA; and ⁷Department of Biochemistry and Molecular Biology, Mayo Clinic Scottsdale, AZ, USA.

**Background:** While circumstantial evidence has associated eosinophils with inflammatory bowel disease (IBD), no studies to date have identified the function of eosinophils in this process. Eosinophils can produce proinflammatory cytokines and granule proteins associated with remodeling. In other non-gastrointestinal (GI) eosinophil related diseases, mechanistic studies support a role for eosinophils in tissue remodeling. The senescence-accelerated (SAMP1) mouse strain develops spontaneous ileitis and recapitulates the pathology of human Crohn’s disease. Further examination of ileal tissue from this strain reveals significant eosinophilic infiltration and remodeling.

**Objectives:** We hypothesize that eosinophilic infiltration to intestinal tissue perpetuates inflammation and remodeling events in IBD. Thus the impact of anti-eosinophil antibody treatment on inflammatory infiltrate and altered tissue architecture in the SAMP1 model of Crohn’s-like ileitis was assessed.

**Methods:** A time-course of histologic and molecular features of ileitis in SAMP1 mice were assessed by routine staining and eosinophil specific immunohistochemical staining. Intestinal permeability was measured by the FITC labeled Dextran gavage method. Eosinophil inhibition studies were performed with a-CCR3 antibody injections during the chronic stage of disease, from 20 to 30 weeks. Flow cytometry for lamina propria leukocytes including eosinophils was performed and all assessments were compared to age-matched control AKR mice.

**Results:** Eosinophilic inflammation correlated with establishment of ileitis and remodeling events in the SAMP1 mouse (p<0.05). Intestinal permeability also increased with disease progression. Blockade of lamina propria eosinophil infiltration was observed following 10-weeks of a-CCR3 injections (p<0.001) and resulted in attenuation of histological measures and ileal inflammation (p<0.05). Molecular measures of remodeling were also reduced. Cellularity of the reactive draining mesenteric lymph nodes, but not the spleen, were significantly reduced (p<0.05). Furthermore, flow cytometric analysis revealed a reduction in lamina propria lymphocyte populations (p<0.05-p<0.001). Reduction in tissue remodeling was also observed, as demonstrated by reduced muscle hypertrophy (p<0.01) and reduced goblet cell hyperplasia (p<0.01). The fibroblast chemo-attractant and eosinophil binding protein fibronectin, was the most increased gene during the progression of remodeling in these mice (p<0.001) and was significantly decreased following antibody treatment (p<0.01).

**Conclusions:** SAMP1 mice demonstrate Crohn’s-like ileitis with histological and molecular features of eosinophilic infiltration and tissue remodeling. Antibody treatment targeting eosinophils are effective in reducing eosinophil infiltration, overall inflammation and tissue remodeling. We anticipate that this model will provide a valuable tool for further elucidating the eosinophil’s role in the pathogenesis of inflammatory bowel diseases.

**Grant Support:** This project was funded by a grant from NIH R01 DK 62245 & NIH R01 DK 080212-01A2, support from the Pappas Foundation, NIH/NCRR Colorado CTSA Grant Number UL1 RR025780 and the NIH CCTSI CMH pilot award (JM). Its contents are the authors’ sole responsibility and do not necessarily represent official NIH views.
POSTER E-9
ROLE OF ANTIMICROBIAL PEPTIDES IN ESOPHAGITIS
Shauna Schroeder, Zachary D. Robinson, Joanne C. Masterson, Lindsay Hosford, Wendy Moore, Sophie A Fillon, Glenn T. Furuta
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Background: Defensins are antimicrobial peptides that contribute to maintaining microbial homeostasis on the surface of the host epithelium. Production of many defensins is constitutive while others are induced by infectious or inflammatory stimuli. An emerging body of data support alterations in the innate defense that contribute to intestinal inflammatory diseases. Eosinophilic esophagitis (EoE) is a chronic disease thought to result from allergenic/immune mediated mechanisms but inciting factors are yet unknown.

Objectives: The overall goal of this study is to measure defensin profile in patients with EoE compared to normal controls. We hypothesize that alterations in defensins contributes to esophagitis in patients with EoE.

Methods: We utilized an in vitro model system (HET-1A esophageal cell line) of EoE to quantify defensin expression. HET cells were grown to 90% confluence with chemokines GMCSF (100 pg/ml) and IL-5 (100 pg/ml) to represent the esophagitis microenvironment in EoE. HET-1A RNA (RNeasy kit, Qiagen) was collected at 24 and 48 hours. Biological relevance was assessed in esophageal mucosal biopsies of well-defined pediatric patients with EoE compared to normal controls. Isolated mRNA was reverse transcribed (cDNA kit, Applied Biosystems) and quantitative RT-PCR performed (Applied Biosystems 7300, Taqman® probes and Absolute Blue® qPCR Master Mix, Thermo Scientific). Sample target expression was normalized to 18S RNA and presented as relative mRNA expression to control (culture system) and fold change (human biopsies).

Results: IL-5 and GMCSF exposure led to decreased hβD1 (human beta defensin) expression at 48 hours and hβD3 at 24 hours compared to HET cells cultured in media alone (hβD1-0.71±0.12 (IL-5,GMCSE) vs 1.05±0.11(control) and hβD3-0.39±0.14 (IL-5,GMCSE) vs 0.95±0.24 (control); p=0.03 and p=0.01, respectively). Esophageal biopsies from EoE patients (n=5) had significantly decreased expression of hβD1 compared to normal (n=11) (0.48±0.86 (EoE) vs 1.1±0.54 (normal); p=0.07). EoE patients treated with topical steroids (n=5) had similar hβD1 expression levels compared to normal (1.3±0.78 (EoE) vs 1.1±0.54 (normal) p=0.76). EoE patients hβD3 expression was significantly decreased compared to normal (0.16±0.21 (EoE) vs 0.93±0.62 (normal); p=0.03) EoE patients treated with topical steroids exhibited hβD3 expression which was significantly reduced compared to normal (0.24±0.17 (EoE) vs 0.93±0.62 (normal); p=0.02). Biopsies from EoE patients have decreased hβD2 expression compared to normal (0.4±0.61(EoE) vs 0.92±0.97 (normal); p=0.29).

Summary: hβD1 and hβD3 are significantly decreased in both in vitro and in vivo models of esophagitis. These decreases in innate immune molecules may potentiate and/or exacerbate esophagitis by decreasing defense barrier function of the esophageal epithelium and modulating the host microbial environment.

Grant Support: University of Colorado Denver School of Medicine Pediatrics Research Track Mentor Program & NASPGHAN mentored summer student research grant (ZR), NIH T32 (SS) and Colorado CTSI. Support by NIH/NCRR Colorado CTSI Grant Number UL1 RR025780. Its contents are the authors’ sole responsibility and do not necessarily represent official NIH views.
EOSINOPHILIC ESOPHAGITIS ASSOCIATED WITH CONGENITAL ESOPHAGEAL ABNORMALITIES AND THEIR REPAIRS

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Background: Eosinophilic esophagitis (EE) is an allergic inflammatory disease that is increasingly diagnosed in pediatric patients in United States and European countries, while few pediatric cases have been reported in Japan. In addition, there are a few reports of eosinophilic esophagitis associated with congenital abnormalities of the esophagus.

Objectives: We hypothesize that the patients who are diagnosed as having eosinophilic esophagitis by the retrospective reviewing of esophageal biopsies can be found and some of them may be associated with congenital or surgery-related stenosis of esophagus.

Methods: The total of 302 upper gastrointestinal endoscopies in our hospital from 2005 to 2009 was viewed focused on the histological findings associated with eosinophilic inflammation. Esophageal biopsy was performed in 20 out of 69 endoscopies (16 patients) for upper gastrointestinal symptoms.

Results: A patient was Barrett’s esophagus, two had Barrett’s mucosa, nonspecific esophagitis were shown in eleven patients and no inflammation was detected in two patients. Among them, there were six patients with eosinophil infiltration. Three out of six patients were histologically proven EE and they were also congenital esophageal stenosis and surgically repaired esophageal atresia and esophageal atresia with esophageal stenosis.

Conclusions: In this study, all patients with histologically proven EE were associated with congenital esophageal abnormalities and their repairs, while no patient was typical of primary EE. The esophageal stricture associated with congenital esophageal abnormalities and their repairs may be involved in the development of EE.


Grant Support: This work is funded by Research on Intractable Diseases, Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan (H22-Nanchi-Ippan-070 to Y.Y.)
POSTER E-11
TREATMENT REVERSES EPITHELIAL MESENCHYMAL TRANSITION IN PEDIATRIC EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) is an increasingly recognized chronic, allergic/immune-mediated disease. Eosinophilic esophageal inflammation leads to remodeling with basal zone hyperplasia, subepithelial fibrosis and in some cases, strictures. Mechanisms leading to subepithelial fibrosis in EoE are poorly defined. Epithelial Mesenchymal Transition (EMT) is a process whereby non-motile epithelial cells de-differentiate into motile mesenchymal-like cells during chronic inflammation, and further differentiate into myofibroblasts. We hypothesized that EMT occurs in EoE and that accepted EoE treatments would reverse this fibrotic phenotype.

Objectives: (1) determine if EMT occurs in children with EoE compared to control subjects, (2) measure the impact of EoE treatment on EMT and its resolution.

Methods: Sections from 60 formalin-fixed, paraffin-embedded esophageal biopsies of patients with EoE (17), GERD (7), indeterminate esophagitis (15) and normal esophagus (21) were evaluated for EMT using three-color immunofluorescence staining for cells expressing cytokeratin (epithelial), vimentin (mesenchymal) and nuclear (DAPI) markers. Patients with EoE were defined using previously published diagnostic guidelines (1); indeterminate esophagitis=clinical features of EoE but eosinophils/HPF < 15; GERD–< 7 eosinophils/HPF (gastroesophageal reflux disease), normal–no eosinophils/esophagitis. Sections were analyzed blinded by two observers using confocal microscopy, and graded for the presence/degree of EMT using a novel 6-point index that assesses the number and location of vimentin positive mesenchymal cells within the hyperplastic epithelium, and the loss of cytokeratin staining. Mean EMT indices/HPF were analyzed for relationships to diagnosis, eosinophils/HPF, and indices for eosinophil peroxidase (EPX) and TGF-b immunostaining, and esophageal fibrosis. Pre- and post-treatment EMT was quantitated in eosophageal biopsies from 18 children with EoE comprising 3 groups: elemental diet (6), empiric six-food elimination diet (6) (SFED) and topical fluticasone (6).

Results: EMT indices were significantly greater in EoE compared to GERD and normal subjects (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>EMT Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>EoE</td>
<td>3.08±0.28</td>
</tr>
<tr>
<td>Indeterminate esophagitis (“EoE”)</td>
<td>2.70±0.29</td>
</tr>
<tr>
<td>GERD</td>
<td>1.71±0.39</td>
</tr>
<tr>
<td>Normal</td>
<td>1.28±0.15</td>
</tr>
</tbody>
</table>

EMT was correlated (all features p<0.01) with eosinophils/hpf (r=0.691), EPX (r=0.738) and TGF-b (r=0.520) staining indices, and eosophageal fibrosis scores (r=0.644). Epithelial vimentin-positive mesenchymal-like cells were a feature of most patients with EoE=12/17 (70.6%) and indeterminate EoE=11/15 (73.3%), compared to one GERD=1/8 (12.5%), and none in normal subjects.

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-treatment EMT Index</th>
<th>Post-treatment EMT Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental diet</td>
<td>3.25±0.76</td>
<td>0.61±0.18 (n=6, p&lt;0.001)</td>
</tr>
<tr>
<td>SFED</td>
<td>3.49±0.71</td>
<td>0.95±0.25 (n=6, p&lt;0.001)</td>
</tr>
<tr>
<td>Topical steroid</td>
<td>2.77±0.92</td>
<td>0.88±0.61 (n=6, p&lt;0.01)</td>
</tr>
</tbody>
</table>

Pre- and post-treatment EMT scores (Table 2) were 3.17±0.82 and 0.82±0.39, respectively for the 18 treated subjects. Resolution of EMT did not differ between the three treatments. Both pre- and post-treatment EMT scores correlated with peak eos/hpf for (elemental diet, r=0.820; SFED, r=0.857; topical corticosteroid, r=0.868; all p<0.001).
Conclusions: Correlations of EMT to esophageal eosinophils, their degranulation and measures of fibrosis suggest that EMT contributes to subepithelial fibrosis characteristic of EoE. Accepted EoE treatments impacting eosinophilia may impact the natural history of EoE and offer long-term benefits in esophageal dysfunction.


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POSTER E-12

THE ESOPHAGEAL STRING TEST (EST): A NOVEL MINIMALLY INVASIVE METHOD FOR MEASURING ESOPHAGEAL INFLAMMATION IN EOSINOPHILIC ESOPHAGITIS

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Background: Endoscopy with biopsy is currently the only method to assess esophageal mucosal inflammation in Eosinophilic Esophagitis (EoE). We hypothesized that the nylon string from the Enterotest™, an existing FDA-approved string-based test used to detect intestinal parasites (Giardiasis), could be used to capture esophageal luminal biomarkers and mediators of inflammation associated with EoE.

Objectives: We sought to determine whether esophageal luminal biomarkers of eosinophilic inflammation as captured by the Esophageal String Test (EST): (1) correlate with those measured in mucosal biopsies, (2) change with disease activity, and (3) differentiate between patients with EoE and gastroesophageal reflux disease (GERD).

Methods: We measured eosinophil-derived granule proteins (EDGPs) in luminal effluents eluted from ESTs and extracts of esophageal biopsies obtained from well-defined children with active EoE (symptomatic with ≥15 eosinophils/HPF and other causes of eosinophilia ruled out), EoE in remission (asymptomatic and <15 eosinophils/HPF), GERD (<15 eosinophil/HPF and/or abnormal pH monitoring) and normal controls (symptomatic, but no esophageal inflammation). ESTs were swallowed the night before endoscopy, removed at endoscopy, placed in elution buffer, centrifuged and supernatants snap-frozen for batch analysis. Esophageal biopsies (EBx) were obtained and snap-frozen after ESTs were removed. Eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), major basic protein (MBP1) and eosinophil peroxidase (EPX) were measured in samples by ELISAs. Statistical analyses compared means using Student’s t test and correlational analyses used Pearson’s test.

Results: ESTs were performed in 22 children (15 males, 11-17 years of age) with active EoE (n=6), EoE in remission (n=4), GERD (n=5) and controls (n=7). EDGPs measured in both the EST and EBx samples correlated with peak eosinophils/hpf for MBP1 (EST: r=0.735, p<.001; EBx r=0.808, p<.001), EDN (EST: r=0.758, p<.001; EBx r=0.830, p<.001), ECP (EST: r=0.606, p<.01; EBx r=0.908, p<.001); similarly, EST and EBx sample EDGPs were significantly correlated with mean eosinophils/HPF. EDGPs measured in EST vs. EBx samples were significantly correlated for MBP1 (r=0.621, p<0.01) and ECP (r=0.436, p=0.05). EDGP levels (ng/ml) from EST samples were greater in active EoE compared to EoE in remission and normal controls (Active EoE vs. EoE remission vs. normal, respectively: EDN=3,372 vs. 803–p=0.109, vs. 577–p=.036; ECP=50,950 vs. 6,236–p=0.223, vs. 8,191–p=0.160; MBP1=16,029 vs. 6,304–p=0.111, vs. 5081–p=.036). EDGP levels in EST samples were greater in children with active EoE compared to GERD respectively (EDN=3,372 vs. 964–p=.092; ECP=50,950 vs. 4,892–p=0.181; MBP1=16,029 vs. 2,237–p=.029).

Conclusions: The EST can measure esophageal mucosal eosinophilic inflammation in children with EoE and can distinguish between active EoE, treated EoE, GERD and normal esophagus in a minimally invasive manner. With further EoE inflammatory biomarker validation, the EST may serve as a minimally invasive surrogate for endoscopy with biopsy to follow disease activity in EoE patients during treatment.

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POSTER E-13
INTERLEUKIN (IL)-15 OVEREXPRESSION IN THE EOSPHAGUS PROMOTES B CELL ACTIVATION AND IMMUNOGLOBULIN CLASS SWITCHING IN EXPERIMENTAL EE.
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Rationale: Our group earlier reported that total IgE, B cells, and immunoglobulin (Ig) isotype switching genes in the esophageal biopsies are induced in human eosinophilic esophagitis (EE); however, the underline mechanism is not well understood. Most recently, we found that IL-15 mRNA and protein is highly expressed in the blood and esophageal biopsies of EE patient. Therefore, we now tested the hypothesis whether increased expression of IL-15 promotes B cell induction, activation and Ig class switching genes in EE.

Methods: The in vitro and in vivo B cell proliferation and activation was measured in IL-15 exposed B cells and IL-15 overexpressed mice by performing flowcytometer or H3 incorporation analysis. The mRNA of IL-15, Ig class switching genes and protein levels were measured by performing real time qPCR and ELISA analysis.

Results: Our analysis indicated that DOX-induced IL-15 overexpression in the mouse esophagus induces B cells, total IgE, IgG1 and IgG2a levels compared to the no DOX exposed rtTA-CC-10-IL-15 transgenic mice. Similarly, we observed induced mRNA levels of Ig class switching genes (GlE, GlG1, GlG2b and GlG3) in the esophagus of DOX exposed IL-15 overexpressed mice compared to no DOX mice. Further, we validated our in vivo data by performing in vitro experimentations using purified splenic B cells from wild type mice. A concentration dependent increase proliferation, activation, and induced Ig class switching genes were observed in purified B cells following recombinant IL-15 treatment.

Conclusions: IL-15 has a critical role in B cell proliferation, activation and esophageal Ig class switching in experimental EE.
POSTER E-14

DISTINCTIVE BLOOD EOSINOPHILIC PHENOTYPES AND CYTOKINE PATTERNS IN EOSINOPHILIC ESOPHAGITIS, INFLAMMATORY BOWEL DISEASE, AND AIRWAY ALLERGY

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Background: Eosinophils are involved in eosinophilic esophagitis (EE) and inflammatory bowel disease (IBD) but their roles herein have been less investigated than in airway allergy.

Objectives: The aim of this study was to examine if blood eosinophil phenotypes differ depending on the underlying eosinophil-associated disorder.

Methods: Adults with symptomatic EE (n=12), IBD (n=9) or airway allergy (n=10), and healthy controls (n=10) were studied with respect to 20 markers on blood eosinophils using 4-color flow cytometry of unfractionated leukocytes. The plasma levels of the eosinophil differentiation factors/chemoattractants Interleukin-2 (IL-2), IL-3, IL-5, GM-CSF, eotaxin and RANTES were measured and all data was processed by multivariate pattern recognition methods to reveal disease-specific clusters.

Results: Patients with EE formed a distinct group with relative eosinophilia, and higher amounts of IL-2, IL-5 and RANTES in plasma. Their eosinophils expressed increased levels of CRTH2, CD11c, CD23, and CD54, and decreased levels of CCR3 compared to healthy and allergic persons. In contrast, eosinophils from IBD patients had diminished surface expression of CD11b, CD18 and CCR3. The eosinophilic phenotypes of airway allergics were indistinguishable from healthy controls, except that IL-2, IL-3, IL-5 and GM-CSF were detectable in their plasma.

Conclusions: The distinct eosinophil phenotypes and plasma cytokines displayed by patients with EE and IBD suggest that circulating eosinophils receive different activation signals from the tissues in these diseases and may therefore acquire different functional properties.

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POSTER F-1
ITCHING RESPONSES AND OTHER INFLAMMATORY EVENTS LINKED WITH DELAYED HYPERSONSITIVITY RESPONSES IN THE SKIN ARE EOSINOPHIL DEPENDENT
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Background: Atopic dermatitis and allergic contact dermatitis are two of the most common skin diseases. In both diseases, eosinophils have been suggested as potential contributors of the pathologies linked with these skin conditions.

Objective: Our goal was to establish a causative relationship between the inflammatory response of chemically induced contact hypersensitivity and eosinophil-mediated activities.

Methods: Wild type and eosinophil-less PHIL mice were sensitized and challenged with 2,4-dinitrochlorobenzene (DNFB) or trimellitic anhydride (TMA). Time lapsed videography was used to capture the number of itching events associated with treatment. Immunohistochemistry for eosinophils and nerves was performed to correlate the effect of dermatitis on innervation and eosinophil accumulation in these mice.

Results: Our studies showed that in both the DNFB and TMA models a robust eosinophil infiltrate occurred that was accompanied by copious levels of eosinophil degranulation. More importantly, these studies demonstrated that metrics of inflammation (e.g., induced ear swelling (i.e., thickness)) was reduced in PHIL mice relative to wild type. Our observations were also directly correlative with a loss of nerve growth and branching in PHIL mice.

Conclusion: These data implicate eosinophils as contributors to the pathologies linked with chemically-induced contact hypersensitivity, including the induced itch responses associated with these models.

This work is supported by the Mayo Foundation for Medical Education and Research.
POSTER F-2
HUMAN EOSINOPHILS DECREASE ALLOGENEIC T CELL PROLIFERATION IN AN IN VITRO MODEL OF GRAFT-VERSUS-HOST DISEASE

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Background: Graft-versus-Host Disease (GvHD) is a complication of allogeneic bone marrow transplantation that may be life-threatening. The acute form of GvHD consists of an exaggerated T cell proliferation, which occurs when donor-derived T cells react against foreign (allogeneic) HLA molecules expressed by tissues of the recipient. Studies have shown an association of elevated levels of eosinophils in peripheral blood and/or tissues with GvHD.

Objectives: In this study we examined whether eosinophilic granulocytes from peripheral blood could alter T cell proliferation in an in vitro model of acute GvHD.

Methods: A mixed lymphocyte reaction (MLR) was created by mixing trigger cells consisting of gamma-irradiated peripheral blood mononuclear cells from healthy donors with freshly isolated responder cells. Eosinophils were purified from blood and added to the cell culture on the 3rd day. Cell proliferation was measured on the 7th day by incorporation of tritiated thymidine or using the CellTrace Violet Cell Proliferation Kit followed by flow cytometric analysis.

Results: Eosinophilic granulocytes decreased allogeneic T cell proliferation in a dose-dependent manner. No such inhibitory effect was seen when neutrophils were used instead of eosinophils. There was no difference in the T cell inhibitory capacity of autologous or heterologous eosinophils.

Conclusions: We have demonstrated that human eosinophils have the ability to down-modulate allogeneic T cell proliferation in vitro. We propose that eosinophils may have an immunoregulatory function in patients afflicted by acute GvHD.

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POSTER F-3

STAPHYLOCOCCUS AUREUS DIRECTLY INDUCES EFFECTOR FUNCTIONS OF EOSINOPHILS IN VITRO

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Background: Eosinophilic inflammation is a cardinal feature of the pathology of atopic dermatitis (AD). Colonization of Staphylococcus aureus (SA) is associated with exacerbation of AD. However, there is little information about the direct interaction of SA and eosinophils.

Objectives: The aim of this study was to investigate the effect of SA on human eosinophils.

Methods: To test whether eosinophils phagocytose SA, FISH-labeled SA was incubated with peripheral blood eosinophils and visualized with confocal laser scanning microscopy. Then, SA-induced eosinophil functions were examined. Eosinophils were cultured with heat-killed SA. First, eosinophil-derived neurotoxin (EDN) release and superoxide generation were measured in the presence or absence of IL-5, IL-17 and IFN-gamma. Then, adhesion of Fluo-3-labeled eosinophils to fibronectin-coated plate was quantified with a fluorescence plate reader. A panel of cytokines released in the supernatants with SA was measured with a multiplex beads array system. Since PAF receptors (PAFR) are reported to be involved in phagocytosis of bacteria by leukocytes, a PAFR blocking peptide was added to eosinophil culture with SA and effector functions above were examined. Results: Eosinophil phagocytosed SA. SA induced significant EDN release and superoxide generation in a dose-dependent manner. IL-5 significantly enhanced SA-induced EDN release. IL-5 and IL-17 significantly enhanced SA-induced superoxide generation, especially in early phase. Eosinophil adhesion to fibronectin-coated plate was significantly enhanced by SA and blocked by anti-CD49d. SA induced a multitude of inflammatory cytokine production from eosinophils, namely, IL-17, IL-6, IL-8, IL-9, MIP-1β, VEGF, FGF basic, IL-1β, TNF-α, IL-12p40/70, MIP-1α, IP-10, and RANTES, but not Th2 cytokines and anti-inflammatory cytokines such as IL-4, IL-5 IL-13, IL-10 and TGF-β. A PAFR blocking peptide significantly inhibited all the functions induced by SA, namely phagocytosis, adhesion to fibronectin, EDN release, superoxide generation, and TNF-α release.

Conclusion: SA directly activates proinflammatory functions of eosinophils through PAFR, suggesting a possible involvement of SA in the pathogenesis of eosinophilic inflammation in AD. PAF blockade may be novel therapeutic approach for AD.

Grant support: This work was supported by a Grant-in-Aid Scientific Research from the Ministry of Health, Labour and Welfare, Japan.
POSTER F-4
TUMOR GROWTH IS ATTENUATED IN EOSINOPHIL-DEFICIENT MOUSE MODELS
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Rationale: Eosinophil infiltration into tumors has been associated with positive and negative growth in human cancers and in mouse models of tumorigenesis. Our previous studies have shown that eosinophils infiltrate tumors and differentially accumulate in the necrotic, capsule and stromal regions of tumors. Using various eosinophil-deficient mouse models, we demonstrate in a solid tumor model that in the absence of eosinophils tumor growth is attenuated.

Objective: To demonstrate using several mouse models of eosinophil-deficiency and eosinophil-mediator deficiency that eosinophils contribute to solid tumor growth.

Methods: Eosinophil migration to tumors was measured by testing inhibitory effects of eosinophil chemoattractants to B16 necrotic media using an ex vivo transwell insert assay system. Tumor growth was assessed using a B16F10 melanoma subcutaneous cell injection model with measurements made on day 12 post-injection. We compared tumor growth in wild type animals vs. various eosinophil-deficient mouse models that are transgenic line of mice (PHIL) and MBP-/-/EPO-/- double knockout mice that are deficient in eosinophil-specific proteins (major basic protein (MBP) and eosinophil peroxidase (EPO)) resulting in a blockade of eosinophilopoiesis. Furthermore, tumor growth was assessed in EPO-/- and MBP-/- animals.

Results: Significantly, ex vivo transwell migration assays using purified eosinophils showed that stressed and/or dying cells elicit robust eosinophil chemotactic response that is inhibited by FTY720 treatment, supporting the hypothesis that this region of tumors is a prominent source of sphingosine-1 phosphate that mediates eosinophil recruitment/accumulation. Tumor growth was decreased by ~40-60% in PHIL and in MBP-/-/EPO-/- double knockout mice relative to tumors in wild type animals. In addition, EPO-/- mice, and not MBP-/-mice, replicate this ~40% reduction in tumor size implicating a significant role for EPO in tumor growth kinetics.

Conclusion: The reduction of tumor size in the absence of eosinophils suggests that eosinophils modulate tumor growth at the tumor site and/or through immune modulating mechanisms. In particular, the reduction of tumor size in EPO-/- mice indicates that one mediator of this pro-tumorigenic activity of eosinophils is eosinophil peroxidase. Although eosinophils are pro-tumorigenic in this study, the role of eosinophils in a variety of tumors may differ due to the unique microenvironment and characteristics of tumor cells.

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