6th biennial symposium

INTERNATIONAL EOSINOPHIL SOCIETY

"EOSINOPHILS 2009"

JULY 7-11, 2009

Hotel Crowne Plaza, Burg, 10, B-8000 Bruges, Belgium

Organizing committee:

Dr. Florence Roufosse
Pr. Monique Capron
Pr. Michel Goldman

Dear Participants,

Welcome to the beautiful city of Bruges, and to the 6th biennial symposium of the International Eosinophil Society!

Previous meetings have proven that excellent science can be conducted on the eosinophil, drawing together scientists and physicians who have chosen to commit themselves to giving this cell the importance it deserves. The convivial meeting atmosphere has contributed to the development of successful partnerships, which have further accelerated our understanding of eosinophil biology. A turning point was reached during the most recent 2007 meeting in Snowbird, as novel and unpredicted roles for the eosinophil emerged. For the **6th biennial meeting**, the scientific program has been broadened to follow-up on these fascinating discoveries. The eosinophil will be positioned at the center of three and a half days of stimulating presentations, high-lighting its role in innate and adaptive immune responses in the setting of infections, allergic diseases, and malignancy, and its ability to communicate with other actors of the immune system. We will also hear what novel mediators and other cell-types have to say for themselves concerning their roles in eosinophil-associated inflammation. In addition to a number of prestigious invited speakers, cutting-edge oral presentations have been selected among a very fine batch of abstracts submitted for this year's meeting. The quality and density of the Eos2009 program proves that eosinophil research is like the big bang, steadily expanding… heralding more excellent meetings in the near future.

Wisdom is pleasurable, but so is Belgian food and hospitality! Although small, Belgium is home to a number of world-renowned specialties and creations: Belgian chocolate, Belgian lace, hundreds of distinctive traditionally brewed ales. Tintin is just one among many of Belgium's comic caracters. Belgians are mocked for their inevitable "French" Fries, but not equaled (Bruges even has a museum dedicated to the Belgian art of the french fry)! To share just a part of what Belgium has to offer in terms of hospitality, we have concocted a very local social program, which should delight congress attendants and accompanying family members. A cocktail will be organized in the historical city center, at the sumptuous Bruges City Hall. On Thursday evening, a paddle steamer will take you to Damme, a nearby medieval town, home to the legendary Thyl Ulenspiegel, whose satirical humour and taste for liberty are considered by some as typical of the Belgian spirit. In Damme, you will be welcomed with a Brueghelian feast, so that you can discover a variety of tasty Belgian specialties. We will end with a dinner party in the modern Concertgebouw, built in 2002 to celebrate Bruge's year as a cultural capital of Europe. Besides these programmed opportunities to sample both ancient and modern Belgian architecture and atmospheres, you will also have one evening on your own, so that you can dine in the smaller taverns which have so much charm. You will have the pleasure of discovering Bruges' canals, its typical houses, which are reminders of the it's past as the richest city of trade in medieval Europe.

We sincerely hope you will enjoy both the scientific and social programs that have been designed with care for your enjoyment, and really encourage you to take some time to discover the charming city which has been chosen to host this year's symposium!

Florence, Monique, and Michel

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ACKNOWLEDGEMENTS

We are deeply grateful for the outstanding financial support of the following corporations, institutions, foundations, societies and individuals:

- CORPORATE SPONSORS -

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- NIH SPONSORS -

National Institute of Allergy and Infectious Diseases
NIH R13 conference grant to Steven Ackerman
Office of Rare Diseases
Conference Grant to Amy Klion for the HES workshop

- FOUNDATION AND SOCIETY SPONSORS -

Fond National de la Recherche Scientifique (Belge)
European Academy of Allergy and Clinical Immunology
INSERM Paris
Institut Pasteur de Lille-IFR 142
Japanese Eosinophil Society

- UNIVERSITIES -

Universiteit Ghent
Université de Lille II
Université Libre de Bruxelles, Faculté de Médecine

ACKNOWLEDGEMENTS

We thank the following individuals for their outstanding fundraising efforts on behalf of the IES and Eosinophils 2009:

Steven Ackerman - Monique Capron - Amy Klion
Florence Roufosse - Hans-Uwe Simon

We thank the scientific committee for diligent evaluation of Eos2009 abstracts:
Steven Ackerman - Amy Klion - Hans-Uwe Simon - Peter Weller

The administrative assistance of the following individuals is gratefully acknowledged:

Bénédicte Meekers - Nicole Willems
Laurence Vilain - Christelle De Beys
Université Libre de Bruxelles, Brussels, Belgium

Jane Whitener
University of Illinois, Chicago

Saurabh Bajpayee
Indian Institute of Technology, Kanpur, India
(Conference website)

Thanks to the following individuals:

Christelle De Beys - Bénédicte Meekers - Florence Roufosse
Université Libre de Bruxelles, Brussels, Belgium
for preparation of the program and abstract book.

Sébastien Coinne
for technical assistance for slide presentations throughout the symposium.

Myriam Libin, Fanny Legrand, and Issam Harfi
for their help in hosting the symposium.

Aline Wybo, from Cardinal Catering,
for expert catering throughout the symposium.

Meeting Brugge
for providing us with precious advice for organization of an event in Bruges, and for supplying brochures about Bruges for symposium participants.

The City of Bruges
for welcoming symposium participants and offering a cocktail in the historical City Hall.

SYMPOSIUM OVERVIEW

Unless otherwise indicated, all activities will take place at the Hotel Crowne Plaza

- General Sessions: *Burgh II-III-V Rooms* **- Posters:** *Arnulf Room* **- Breaks:** *Foyer*
Breakfast: *Restaurant De Linde* **– Lunch:** *St Donaas and Oostpoort rooms*

Tuesday, July 7th 2009

17.00 Registration Opens in the Foyer, Hotel Crowne Plaza

19.30 ***Welcome and "Get Together" dinner*** *at the Hotel Crowne Plaza (St Donaas Room)*

Wednesday, July 8th 2009

8.00 **Session 1**
Regulation of eosinophil development, functional maturation, and death

10.30 **Session 2**
Eosinophil functions: trafficking, activation, and signaling

12.30 *Lunch*

13.30 **Session 3**
The eosinophil: A central player in innate immunity?

16.30 **Session 4**
Non-eosinophil cell populations sharing characteristics with eosinophils

18.30 ***Cocktail at Bruges City Hall***

20.00 *Unprogrammed Dinner (assistance with dinner reservations provided if needed)*

Thursday, July 9th 2009

8.00 **Session 5**
Eosinophil / T cell interactions in health and disease

10.15 **Session 6**
Novel findings on cytokines, chemokines and lipid mediators involved in eosinophil biology and eosinophil-associated disorders

12.15 **3rd Ehrlich Lectureship and Award Presentation**

13.00 *Unscheduled free time*

15.45 **Session 7**
Eosinophils and Disease (I): Allergic Inflammation

19.00 ***Breughelian Feast in Damme*** *(cocktail on Paddle Boat)*

Friday, July 10th 2009

8.00 **Session 8**
Induction/ Regulation of Eosinophil-Mediated Damage, Repair, Remodeling and Fibrosis

10.15 **Back to back presentations**

12.00 *Unscheduled free time*

15.00 **Poster Viewing and Discussion**

17.00 **Session 9**
Eosinophils and Disease (II): Malignancy, Parasitosis and others

19.30 ***Dinner and dancing at the Concert Gebouw*** *(Presentation of Junior Investigator Travel Awards)*

Saturday, July 11th 2009

8.00 **Session 10**
Existing Treatment Strategies and Targets for Eosinophil-Mediated Disease

10.30 **Session 11**
New Therapeutic Targets for Eosinophil-mediated Disease

12.00 *Acknowledgments and Adjournment*

12.30 **Meeting of the IES Executive Committee**

IN CASE OF EMERGENCY
Should you need to reach a member of the symposium organizing group during your stay at Bruges, please note the following cell phone numbers:
Florence Roufosse: +32/477 18 43 11
Nicole Willems: +32/475 74 09 57
Bénédicte Meekers: +32/476 98 83 72

PROGRAM

Tuesday, July 7th 2009

17:00 Registration Opens in the **Foyer, Hotel Crowne Plaza**

19:30-22:00 **Welcome and "Get Together" dinner at the Hotel Crowne Plaza** (St Donaas Room)
Florence Roufosse (Université Libre de Bruxelles, Belgium), Monique Capron (University Lille II, France), Michel Goldman (Université Libre de Bruxelles, Belgium), Meeting Organizers
Steven Ackerman (University of Illinois, Chicago College of Medicine, United States), IES President and Treasurer

NOTE THAT ALL GENERAL SESSIONS THROUGHOUT THE SYMPOSIUM WILL BE HELD IN THE BURGH II, III AND V ROOMS

Wednesday, July 8th 2009

8:00-10:00 **Session 1: Regulation of eosinophil development, functional maturation, and death**
Moderators: James Lee (Mayo Clinic Arizona, United States) and Judah Denburg (McMaster University, Ontario, Canada)

8:00 **State-of-the-Art:** Steven Ackerman (University of Illinois, Chicago College of Medicine, United States)
Transcriptional regulation of eosinophil development

8:30 Oral "Cutting Edge" abstract: Martijn Nolte (Academic Medical Center, Amsterdam, Netherlands)
T-cell derived IFN-γ inhibits eosinophil differentiation

8:45 Oral "Cutting Edge" abstract: Brian Maybruck (University of Illinois) Lentiviral shRNA Knockdown of Charcot-Leyden Crystal Protein/Galectin-10 Expression in Developing Human Eosinophils Impairs Granulogenesis

9:00 **State-of-the-Art:** Chris Haslett (University of Edinburgh, United Kingdom) Mechanisms involved in resolution of granulocyte-mediated inflammation

9:30 Invited Speaker: James Malter (University of Wisconsin, United States) Mechanisms of eosinophil activation and survival in vitro and in vivo

9:45 Invited Speaker: Leo Koenderman (University Medical Center Utrecht, Netherlands)
GM-CSF-induced survival in eosinophils is not mediated via beta-common (CD131)

10:00 *Coffee break*

10:30-12:30 **Session 2**: **Eosinophil functions (trafficking, activation, and signaling)**
Moderators: Andrew Wardlaw (University of Leicester, United Kingdom) and James Malter (University of Wisconsin, United States)

10:30 **State-of-the-Art:** Joan Cook-Mills (Northwestern University, Chicago, United States)
Eosinophil recruitment: Endothelial cell function and beyond

PROGRAM

Wednesday, July 8th 2009

11:00	Oral "Cutting Edge" abstract: Leticia Lintomen (State University of Campinas, Brazil) The effect of obesity on eosinophilic infiltration in a murine model of allergic asthma
11:15	**State-of-the-Art:** Peter Weller (Beth Isreal Deaconess, Harvard Medical School, Massachusetts, United States) Overview of mechanisms regulating eosinophil mediator secretion
11:45	Invited Speaker: Jan Tavernier (Universiteit Ghent, Belgium) The MAPPIT toolbox: strategies to analyze molecular interactions in intact cells
12:00	Oral "Cutting Edge" abstract: Paige Lacy (University of Alberta, Canada) Agonist activation of eosinophil shape change and mediator release is dependent on RAC2 GTPase
12:15	Oral "Cutting Edge" abstract: Ester Boix (Universitat Autonoma de Barcelona, Spain) The N-terminus of the eosinophil cationic protein (ECP) retains most of its bactericidal and membrane disruption capacities
12:30	*Lunch (provided for symposium registrants at the Crowne Plaza Hotel)*
13:30-15:45	**Session 3**: **The eosinophil: A central player in innate immunity?** Moderators: Hirohito Kita (Mayo Clinic Rochester, Minnesota, United States) and Hans Uwe Simon (University of Bern, Switzerland)
13:30	**State-of-the-Art:** Helene Rosenberg (NIAID, National Institutes of Health, Maryland, United States) Eosinophil-mediated modulation of innate immune responses to viral infection
14:00	Invited Speaker: Monique Capron (University Lille II, France) Human eosinophils express a functional γδTCR/CD3 complex: role in innate responses against mycobacteria and tumors
14:15	Invited Speaker: Jens Ponikau (University at Buffalo, New York, United States) Eosinophils: The killing machinery against fungi
14:30	Invited speaker: Darryl Adamko (University of Alberta, Canada) Virus-induced release of eosinophil mediators and their role in asthma attacks
14:45	**State-of-the-Art:** Hans-Uwe Simon (University of Bern, Switzerland) Eosinophils and host defense against bacteria
15:15	Invited Speaker: Christine Wenneras (Sahlgrenska University Hospital, Sweden) Bacteria and eosinophil activation
15:30	Oral "Cutting Edge" abstract: Elizabeth Bivins-Smith (Oregon Health & Science University, United States) Eosinophils mediate antiviral effects in vivo and directly kill parainfluenza virus in vitro
15:45	Oral "Cutting Edge" abstract: Stanislaw Gabryszewski (NIAID, Maryland, United States) The innate immune shield: probiotic lactobacillus species promote expression of eosinophil-active cytokines and resistance to the lethal sequelae of respiratory virus infection
16:00-16:30	*Coffee break*

PROGRAM

Wednesday, July 8th 2009

16:30-17:30 **Session 4**: **Non-eosinophil cell populations sharing characteristics with eosinophils**
Moderators: Monique Capron (University Lille II, France) and Redwan Moqbel (University of Manitoba, Canada)

16:30 **State-of-the-Art**: Hajime Karasuyama (Tokyo Medical and dental University, Japan) - Newly discovered roles for basophils: a neglected minority gains new respect

17:00 Invited Speaker: Javier Monteseirin (Universidad de Sevilla, Spain) - Neutrophils and ECP

17:15 Invited Speaker: Matthias Mack (University of Regensburg, Germany) - Role of basophils in immunological memory responses

18:30 ***Cocktail at Bruges City Hall***

Thursday, July 9th 2009

8:00-9:45 **Session 5**: **Eosinophil / T cell interactions in health and disease**
Moderators: Michel Goldman (Université Libre de Bruxelles, Belgium) and Paul Foster (University of Newcastle, Australia)

8:00 **State-of-the-Art**: James J. Lee (Mayo Clinic Arizona, United States)
Eosinophils in health and disease: The LIAR hypothesis

8:30 Invited speaker: Hiroshi Nakajima (Chiba University, Japan)
Th2, Th17, and allergic inflammation

8:45 Oral "Cutting Edge" abstract: Elizabeth Jacobsen (Mayo Clinic Arizona, United States)
Site-specific reconstitution of eosinophils in eosinophil-deficient (PHIL) mice differentially affects T cell activation and recruitment in allergic pulmonary inflammation

9:00 Invited Speaker: Paul Foster (University of Newcastle, Australia)
The role of small non-coding (micro) RNAs in the modulation of eosinophilia and Th2 regulated inflammation: a new therapeutic approach to treat asthma

9:15 Invited speaker: Clare Lloyd (Imperial College, London)
Regulation of allergic inflammation and AHR by T cell subsets

9:30 Oral "Cutting Edge" abstract: Haibin Wang (Beth Israel Deaconess, Massachusetts, United States)
Eosinophils inhibit TGF-beta-induced Foxp3+ regulatory CD4+ T cells by releasing IL-4

9:45-10:15 *Coffee break*

PROGRAM

Thursday, July 9th 2009

10:15-12:15 **Session 6**: **Novel findings on cytokines, chemokines, and other mediators involved in eosinophil biology and eosinophil-associated disorders**
Moderators: Peter Weller (Beth Israel Deaconess, Harvard Medical School, Massachusetts, United States) and Marc Rothenberg (Cincinnati Children's Hospital Medical Center, Ohio, United States)

10:15 **State-of-the-Art:** Yui Hsi Wang (Cincinnati Children's Hospital Medical Center, Ohio, United States)
Involvement of TSLP, OX40-L, and IL-25 in atopic disorders

10:45 Invited Speaker: Roy Pettipher (Oxagen Ltd, Oxon, United Kingdom)
Involvement of CRTH2 in eosinophilic inflammation - basic biology and clinical findings

11:00 Invited Speaker: Hirohito Kita (Mayo Clinic Rochester, Minnesota, United States)
Roles of epithelial alarmin IL-33 in eosinophilic inflammation

11:15 Invited Speaker: Jean-Christophe Renauld (University Catholique de Louvain, Belgium)
Involvement of IL-9 in allergy and parasitosis

11:30 Invited speaker: Hans-Erik Claesson (Karolinska Institute, Sweden)
Eoxins: novel proinflammatory arachidonic acid metabolites produced by human eosinophils

11:45 Oral "Cutting Edge" abstract: Maria Lampinen (University Hospital Upssala, Sweden)
Human eosinophils express muscarinic receptors, produce corticotropin releasing hormone in the colon and may participate in the regulation of macromolecular permeability via mast cells

12:00 Oral "Cutting Edge" abstract: Josiane Neves (Harvard Medical School, Massachusetts, United States)
Cysteinyl leukotrienes elicit secretion by human eosinophil granules

12:15-13:00 **3rd Ehrlich Lectureship Award Presentation and Lecture** – Professor Redwan Moqbel (University of Manitoba, Canada)

13:00-15:45 *Unscheduled free time*

15:45-17:45 **Session 7**: **Eosinophils and Disease (I): Allergic Inflammation**
Moderator: Robert Schleimer (Northwestern University Feinberg School of Medicine, Illinois, United States)

15:45 **State-of-the-Art:** Marc Rothenberg (Cincinnati Children's Hospital Medical Center, Ohio, United States)
Mechanistic Dissection of Eosinophilic Gastrointestinal Disorders (EGID)

16:15 Invited Speaker: Alex Straumann (Kantonsspital Olten, Switzerland)
Eosinophilic esophagitis: an omnium gatherum, a variant of GERD, or a specific disease?

16:30 Invited Speaker: Gerald Gleich (University of Utah, United States)
Novel mechanisms of eosinophil granule protein induced skin lesion formation

16:45 Invited Speaker: Judah Denburg (McMaster University, Ontario, Canada)
Cord blood eosinophil progenitor phenotypes and risks for development of atopy and asthma in early life

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Thursday, July 9th 2009

17:00 Invited Speaker: Steven Varga (University of Iowa, United States)
Contribution of eosinophils to RSV vaccine-enhanced disease

17:15 Oral "Cutting Edge" abstract: Akira Kanda (Institut Pasteur de Lille, France)
Eosinophil-derived IFN-gamma induces airway hyperresponsiveness and lung inflammation in the absence of lymphocytes

17:30 Oral "Cutting Edge" abstract: Anil Mishra (Cincinnati Children's Hospital Medical Center, Ohio, United States)
Key role of interleukin-15 in the pathogenesis of eosinophilic esophagitis

19:00-23:00 ***Breughelian Feast in Damme*** *(bus pick-up at hotel - cocktail on Paddle Boat)*

Friday, July 10th 2009

8:00-9:45 **Session 8: Induction/Regulation of Eosinophil-Mediated Damage, Repair, Remodeling and Fibrosis**
Moderators: Barry Kay (Imperial College, London, United Kingdom) and Seema Aceves (Children's Hospital, San Diego, United States)

8:00 **State-of-the-Art:** Francesca Levi-Schaffer (Hebrew University of Jerusalem, Israel)
Eosinophils and angiogenesis: Update on mechanisms involved in eosinophil-associated tissue remodeling

8:30 Invited Speaker: Seema Aceves (Rady Children's Hospital, San Diego, United States)
Mechanisms of eosinophil-mediated tissue remodeling responses in eosinophilic esophagitis

8:45 **State-of-the-Art:** Allison Fryer (Oregon Health and Science University, United States)
Eosinophil-induced damage to parasympathetic nerves

9:15 Invited Speaker: Lu-Yuan Lee (University of Kentucky Medical Center, United States)
Airway hypersensitvity induced by eosinophil granule-derived cationic protein

9:30 Oral "Cutting Edge" abstract: Erin Fitch (Oregon Health & Science University, United States)
Interactions between eosinophils and nerves in atopic dermatitis

9:45-10:15 *Coffee break*

10:15-12:00 **Back to back presentations**

I. *Relationships between eosinophilia, host defense against infectious agents, and allergy: Do they tell us anything?*
Moderators: Thomas Nutman (National Institutes of Health, United States) and André Capron (Académies des Sciences, Institut de france, Paris)

Padraic Fallon (Trinity College, Dublin, Ireland) - Helminth modulation of allergic inflammation: novel mechanisms and molecules
Bart Lambrecht (Universiteit Ghent, Belgium) - The function of dendritic cell subsets in allergic asthma and influenza

PROGRAM

Friday, July 10th 2009

II. *Mechanisms of eosinophil-associated inflammatory bowel disease : From mouse models to man.*
Moderator: Alex Straumann (Kantonsspital Olten, Switzerland)

Glenn Furuta (University of Colorado, United States) - Eosinophils in inflammatory bowel disease
Simon Hogan (University of Cincinatti, United States) - Resident intestinal macrophage regulation of eosinophil inflammation in IBD

12:00-15:00 *Unscheduled free time*

15:00-17:00 **Poster Viewing and Discussion** (Hotel Crowne Plaza, Salle Arnulf)

17:00-18:45 **Session 9**: **Eosinophils and Disease (II): Malignancy, Parasitosis, and others.**
Moderators: Francesca Levi-Schaffer (Hebrew University of Jerusalem, Israel) and Jean-Christophe Renauld (University Catholique de Louvain, Belgium)

17:00 **State-of-the-Art:** Michael Lotze (University of Pittsburgh School of Life Sciences, United States)
You eat what you were: Eos, DAMPS, and cancer

17:30 Invited Speaker: Judy Appleton (Cornell University, New York, United States
Eosinophil deficiency compromises parasite survival in chronic nematode infection

17:45 Invited speaker: Mike Wechsler (Brigham and Women's Hospital, Harvard Medical School, Massachusetts, United States)
Novelties in Churg-Strauss Syndrome diagnosis and management

18:00 Invited speaker: Per Venge (Uppsala Academic Hospital, Sweden)
What do genomics and proteomics tell us about the role of eosinophils in disease?

18:15 Oral "Cutting Edge" abstract: Katrin Gentil (University Hospital Bonn, Germany)
A key role for eotaxin-1 in antifilarial immunity via regulation of eosinophil activation

18:30 Oral "Cutting Edge" abstract: Steve Maltby (Biomedical Research Center, Vancouver, Canada)
CD34 is required for the infiltration of inflammatory cells into the mouse colon during DSS-induced colitis

19:30-23:00 ***Dinner and dancing at the Concert Gebouw***
Presentation of Junior Investigator Travel Awards – Steven Ackerman, IES President and Treasurer

PROGRAM

Saturday, July 11th 2009

8:00-10:15	**Session 10: Existing Treatment Strategies and Targets for Eosinophil-Mediated Disease** Moderators: Amy Klion (National Institutes of Health, Maryland, United States) and Gerald Gleich (University of Utah, United States)
8:00	**State-of-the-Art:** Jan Cools (Katholieke Universiteit Leuven, Belgium) Update on tyrosine kinase inhibitors in chronic eosinophilic leukemia
8:30	Invited Speaker: Robert Schleimer (Northwestern University Feinberg School of Medicine, Illinois, United States) Chronic rhinosinusitis as a cardinal eosinophilic disease of the human airways
8:45	Invited speaker: Kris Leiferman (University of Utah, United States) Pathogenesis of eosinophilic skin disorders
9:00	**State-of-the-Art:** Parameswaran Nair (McMaster University, Ontario, Canada) Lessons learned from anti-IL-5 clinical trials in "eosinophilic" asthma
9:30	Invited Speaker: Andrew Wardlaw (University of Leicester, United Kingdom) Treatment with a monoclonal antibody against IL-5 (mepolizumab) reduces severe exacerbations in people with refractory asthma
9:45	Oral "Cutting Edge" abstract: Florence Roufosse (Université Libre de Bruxelles, Belgium) Response to mepolizumab in patients with T cell mediated HES
10:00	*Coffee break*
10:30-12:00	**Session 11: New Therapeutic Targets for Eosinophil-mediated Disease** Moderators: Florence Roufosse (Université Libre de Bruxelles, Belgium) and Bruce Bochner (Johns Hopkins University, United States)
10:30	**State-of-the-Art:** Amy Klion (National Institutes of Health, Maryland, United States) Targeted immune-based therapies for the treatment of eosinophilic disorders
11:00	Invited Speaker: Carinne Blanchard (Cincinnati Children's Hospital, Ohio, United States) Novel therapeutic targets in EGIDs
11:15	Invited speaker: Sameer Mathur (University of Wisconsin, United States) Anti-IL-5R antibodies for eosinophil-associated diseases
11:30	Invited speaker: Bruce Bochner (Johns Hopkins University, United States) Siglecs and other inhibitory receptors on eosinophils as potential therapeutic targets
11:45	Oral "Cutting Edge" abstract: Els Lierman (Katholieke Universiteit Leuven, Belgium) Downstream effectors of FIP1L1-PDGFRa as targets for therapy in chronic eosinophilic leukemia
12:00	**Acknowledgements and adjournment –** Florence Roufosse, Monique Capron, Michel Goldman, Steven Ackerman
12:30 - 15:30	*Meeting of the IES Executive Committee (with lunch)*

EHRLICH LECTURESHIP

PAUL EHRLICH LECTURESHIP

INTERNATIONAL EOSINOPHIL SOCIETY

2009

The Ehrlich Lectureship is awarded at the biennial eosinophil symposia of the International Eosinophil Society 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.

Prior honourees of the International Eosinophil Society, recognized for these types of contributions, prior to formal establishment of the Ehrlich Lectureship in 2005, include:

Honored at Eosinophils 2001, Banff, Alberta Canada:

Dr. Monique Capron
Dr. Ann M. Dvorak
Dr. Gerald J. Gleich
Dr. A. Barry Kay
Dr. Per Venge

Past Recipients of the Ehrlich Lectureship:

Dr. Christopher J.F., Spry (Eosinophils 2005, Bern Switzerland)
Dr. Colin Sanderson (Eosinophils 2007, Snowbird UT, USA)
Dr. Kyoshi Takatsu (Eosinophils 2007, Snowbird UT, USA)

Recipient of the 2009 Ehrlich Lectureship:

Dr. Redwan Moqbel, Ph.D., FRCPath

PAUL EHRLICH LECTURESHIP

INTERNATIONAL EOSINOPHIL SOCIETY

2009

Redwan Moqbel, Ph.D., FRCPath

For your outstanding contributions to our understanding of eosinophil cell biology, immunobiology and mechanisms of eosinophil secretion

Dr. Redwan Moqbel is an international authority on the immunobiology of human eosinophils and their potential role in airway disease. He started his interest in the eosinophil as early as 1976 during his PhD studies at the University of London's School of Hygiene and Tropical Medicine, working on nematode immunity and associated inflammation. He then joined Professor A. Barry Kay's laboratory at the National Heart and Lung Institute in London, UK, between 1980 and 1995, where he made seminal observations about the capacity of human eosinophils to synthesize, store and release cytokines, chemokines and growth factors. He was a member of the team that published the first report about eotaxin, and was involved in numerous studies on the role of the eosinophil in asthma and related allergic diseases. He was recruited to the University of Alberta in Edmonton, Canada, in 1995. His research there contributed to our current understanding of the complex intracellular exocytotic mechanisms regulating human eosinophil mediator release as well as the role of the eosinophil in regulating the Th2 immune response via tryptophan catabolism. Redwan was a founding member of the International Eosinophil Society; he served as IES President between 2003-2005. He was the Co-Organizer of two successful Eosinophil Symposia, the first in Rio de Janeiro, Brazil, in 1996 and the second, the biennial International Eosinophil Symposium of the IES, in Banff, Alberta, Canada in 2001. Redwan was the Director of the Pulmonary Research Group at the University of Alberta in Edmonton and is currently the Chair of the Department of Immunology at the University of Manitoba in Winnipeg. He is an ardent supporter and an active promoter of the eosinophil and its significance in immunity and inflammation.

INVITED SPEAKER ABSTRACTS

REGULATION OF EOSINOPHIL DEVELOPMENT: STATE-OF-THE-ART

Steven J. Ackerman

Department of Biochemistry & Molecular Genetics, University of Illinois at Chicago, USA.

Studies of the transcriptional mechanisms that regulate myeloid gene expression, hematopoietic lineage specification and development have provided new insights into the roles of combinatorial networks of transcription factors in determining progenitor cell fate. Findings from avian, mouse and human studies demonstrate that a handful of transcription factors and their functional interactions are critical to the specification of the eosinophil lineage. The combinatorial activities of GATA-1, C/EBPα and PU.1 are required for eosinophil development, with GATA-1 the pivotal factor determining whether common myeloid progenitors (CMPs) differentiate into eosinophils (requires GATA-1) or neutrophils and macrophages (absence of GATA-1). FOG-1, a co-activator of GATA-1 in the erythroid lineage, acts as a co-repressor that antagonizes GATA-1 activity in eosinophil progenitors, and it must be down regulated for eosinophil development to proceed. Mouse knockout studies show that eosinophils do not develop in GATA-1 null mice, and that transgenic deletion of a high-affinity double GATA site in the mouse GATA-1 regulatory locus results in a lineage-specific block in eosinophil differentiation, indicating these double GATA sites regulate eosinophil lineage-specific expression of GATA-1. Consonant with this is our identification of these high-affinity double GATA sites in the promoters of a number of genes that define the eosinophil lineage, including the secondary granule proteins MBP1 and EPX, the CLC protein (galectin-10), receptors such as IL-5Rα, eotaxin receptor CCR3, and the transcription factor C/EBPe. In contrast to the antagonistic activities of GATA-1 and PU.1 functionally defined in erythroid vs. myeloid differentiation, a process thought to resolve lineage promiscuity as part of progenitor cell fate specification during hematopoiesis, PU.1 and GATA-1 synergize in the eosinophil lineage for transcription of genes such as MBP1. The mechanism for PU.1-GATA-1 synergy appears to involve PU.1 enhancement rather than antagonism of GATA-1 binding to the high affinity double GATA sites in many of these key eosinophil genes. Once CMPs express the IL-5R, they are now considered committed to the eosinophil lineage, and their terminal differentiation requires the activity of C/EBPε, expressed in human eosinophils as four different temporally regulated isoforms, two activators and two alternatively spliced repressors, that together coordinately regulate eosinophil gene transcription and progenitor development. C/EBPε knockout mice lack terminally differentiated eosinophils (and neutrophils). Similarly, patients with specific granule deficiency (SGD) have a loss-of-function mutation in the C/EBPε gene, with failure of eosinophil and neutrophil terminal differentiation including failed expression of secondary granule protein genes in both granulocytes.

Greater understanding of the combinatorial interactions of the transcription factors and their co-activators/co-repressors that specify the eosinophil's developmental program and regulate gene transcription and differentiation is still needed, as are studies of epigenetics and non-coding, e.g. micro, RNAs. Future studies may identify novel transcriptional targets for specifically ablating eosinophil development or knocking out expression of key mediators, e.g. granule proteins or receptors such as CCR3, as therapeutic approaches to the treatment of eosinophil-mediated allergic diseases and hypereosinophilic syndromes.

Dr. Ackerman is funded by grants from the NIH/NIAID, AGA and the CURED Foundation.

INVITED SPEAKER ABSTRACTS

MECHANISMS INVOLVED IN RESOLUTION OF GRANULOCYTE-MEDIATED INFLAMMATION

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Inflammatory diseases are responsible for a heavy burden of morbidity and untimely deaths, particularly in the developed world. For many of these, current treatments are poorly effective, associated with major adverse consequences, or both.

Much attention has been focused on agents that might interfere with the initiation processes of inflammation. Our approach has been different. The guiding hypothesis has been that identification of the processes by which even dramatic inflammatory responses, such as those seen in streptococcal pneumonia and acute gout, can resolve completely will lead to a new approach to therapy based on 'driving' these natural resolution mechanisms.

One pre-requisite for inflammatory resolution is that extravasated granulocytes and their 'payloads' of potentially histotoxic granule contents must be efficiently removed. Having shown that neutrophils undergo apoptosis, a process responsible for shutting-down of secretory responses and removal of the intact cell and its contents by inflammatory macrophages utilising unique recognition mechanisms that fail to incite a pro-inflammatory response, we have focused on harnessing this process for therapeutic benefit.

The apoptotic 'machinery' is available to both neutrophilic and eosinophilic granulocytes, although different longevity controls appear to be operating. We have identified a number of agents which differentially drive apoptosis in neutrophils and eosinophils in vitro, but their effects in vivo appear to be overridden by powerful factors like GM-CSF.

Recently, however, we have found that R-roscovitine and other cyclin inhibitors powerfully induce neutrophil apoptosis in vitro and in vivo in association with greatly accelerated resolution of inflammatory/scarring lesions in the lung and joints.

INVITED SPEAKER ABSTRACTS

PIN1 REGULATES PROSURVIVAL CYTOKINE SIGNALING IN EOSINOPHILS

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Background: Eosinophils (Eos) become long-lived effector cells after exposure to IL-5 or GM-CSF, a process that can be disrupted by steroids. Prosurvival signaling inactivates Bax, a Bcl-2 homolog that is abundantly expressed by Eos. How prosurvival signaling prevents Eos apoptosis is unclear.

Objectives: To identify the mechanisms elicited by IL-5/GM-CSF that promote Eos survival.
Methods: Peripheral blood Eos were obtained from healthy donors and evaluated for apoptosis by flow after 2-3 days in culture in the presence of IL-5/GM-CSF with or without Pin1, Erk, calpain, caspase, PKCα or PP2A inhibitors. Immunoprecipitation/western blotting (IP/WB) was performed to identify interactions between proteins and their phospho-status. Immunofluorescence was performed to follow Bax translocation.

Results: IL-5 or GM-CSF significantly prolonged Eos survival which was prevented by inhibitors of Pin1, Erk, PP2A or PKCα Conversely, the survival of untreated Eos were prolonged by inhibitors of caspases, calpain or Bax. Prosurvival signaling rapidly increased Pin1 isomerase and Erk activity, leading to phosphorylation of Bax on Thr167. Phospho-Bax interacted with active Pin1. In the absence of Pin1 activity, Erk activity or the presence of mutant Bax (Thr167 converted to Ala167), prosurvival signaling was suppressed and apoptosis ensued. Calpain cleaved Bax, facilitating its translocation to and disruption of mitochondria.

Conclusions: Prosurvival signaling suppresses Bax activation by inducing Erk mediated, Thr167 phosphorylation. Pin1 binds to phospho-Bax and constrains its activation, likely by isomerization of the Thr167-Pro168 bond. In the absence of prosurvival signaling, Eos rapidly undergo Bax activation and apoptotic cell death. By accelerating Eos apoptosis, Pin1 inhibitors may have clinical utility in asthma and other eosinophilic diseases.

This project was funded by grants from NIH (P01HL088594 and R01HL087950).

GM-CSF-INDUCED SURVIVAL OF EOSINOPHILS IS NOT MEDIATED VIA β-COMMON (CD131)

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Background: b-common (CD131) containing receptors (GM-CSFR, IL-3R and IL-5R) are potent inducers of survival in eosinophils as well as cell lines that are dependent on the ligands of these receptors. A body of evidence points at the importance of b-common in survival signaling as tyrosine mutants of these receptors is deficient in responding to these cytokines in terms of postponing apoptosis. However, interpretation of the experiments carried out with cell lines is complicated by the fact that proliferation is also inhibited by mutation of the b-common chain. A block in proliferation might induce apoptosis irrespective of signaling mediated by the b-common chain.

Objectives: To evaluate the role of the β-common chain and GMR-alpha chain in GM-CSF-induced survival in primary terminally differentiated eosinophils and GMR-expressing Ba/F3 cells.

Methods: Eosinophils were isolated from normal the peripheral blood of normal donors. These cells were cultured in vitro in the presence and absence of 3T3 fibroblasts. We applied the GM-CSF mutant E21R-GM-CSF which can only bind to the alpha-chain and, therefore, functions as a potent antagonist of the GM-CSFR. Interestingly, it can by itself modulate eosinophil survival pointing at a role of GMR-α. In addition, we constructed Ba/F3 cells with different GM-CSFR mutants and cells only expressing GMR-α-chain. The experiments were performed with unprimed cells or cells pre-activated with GM-CSF and IL-5. Apoptosis was measured by flowcytometry applying annexin-V.

Results: A complete block of signaling through the β-common chain as visualized by the inability of GM-CSF to induce phosphorylation of ERK1/2 as well as p38-MAPK was associated with normal GM-CSF and IL-5-induced survival of eosinophils. In fact, antagonism of β□common induced a small but significant survival in unprimed cells. Priming of eosinophils with GM-CSF dramatically change this behavior as blocking of β-common function induced clear apoptosis in these cells. The same phenotype was found in Ba/F3 cells only expressing the β-chain of the GM-CSFR.

Conclusions: The data are consistent with the hypothesis that in terminally differentiated eosinophils the β-common chain is not involved in survival signaling induced by GM-CSF. The β-chain seems to induce a death signal by itself by (a) yet to be defined mechanism(s) that needs to be confined in order to allow survival of these cells.

This project was funded by a grant from the UMC-Utrecht.

EOSINOPHIL RECRUITMENT: ENDOTHELIAL CELL FUNCTION AND BEYOND

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Background: The prevalence of asthma in several countries has dramatically increased in the last 40 years. Thus, it is critical to determine mechanisms of asthma in order to identify novel targets for intervention in this disease. A hallmark of allergic asthma is eosinophilia. The recruitment of eosinophils is dependent on the endothelial cell adhesion molecule VCAM-1. Our research has overturned the paradigm that VCAM-1 on the endothelial cell surface is simply a scaffold for leukocyte migration. We discovered that VCAM-1 activates a signaling cascade in endothelial cells through: calcium, Rac-1, NOX2, reactive oxygen species, MMPs, PKCα and PTP1B. These signals are required for VCAM-1-dependent leukocyte transendothelial migration in vitro. We have examined whether VCAM-1 signals regulate eosinophil recruitment in vivo.

Objective: Determine whether VCAM-1 signals in endothelial cells regulate eosinophil recruitment in vivo and whether this eosinophilia is modulated by isoforms of the antioxidant vitamin E.

Methods: VCAM-1-dependent migration of leukocytes was examined in vitro under physiological laminar flow. VCAM-1-dependent eosinophil recruitment in experimental asthma was examined in mice deficient in VCAM-1 signaling intermediates. To address antioxidant regulation of eosinophilia, mice were administered α-tocopherol and/or γ-tocopherol isoforms of vitamin E after sensitization but before OVA challenge. Inflammation and airway responsiveness was examined.

Results: In response to OVA challenge, non-hematopoietic NOX2 deficient mice had increased numbers of eosinophils bound to the luminal surface of the endothelium as well as reduced eosinophilia in the lung tissue and bronchoalveolar lavage. This occurred independent of changes in numbers of T cells, neutrophils or mononuclear cells in the lavage fluids and without changes in VCAM expression, cytokines or chemokines. For vitamin E-treated BALB/c mice, α-tocopherol inhibited eosinophilia whereas, surprisingly, γ-tocopherol elevated eosinophilia. Moreover, γ-tocopherol ablated the benefit of α-tocopherol. A mechanism for these opposing immunoregulatory functions of purified tocopherols at physiological concentrations is not through modulation of lung expression of several cytokines, chemokines or adhesion molecules but is, at least in part, by regulation of endothelial cell signals for leukocyte recruitment.

Conclusions: VCAM-1 signaling in the endothelium is necessary for the eosinophil recruitment during allergic inflammation. During this eosinophil recruitment, isoforms of tocopherols have opposing immunoregulatory functions. Moreover, the proinflammatory function of the d-γ-tocopherol isoform of vitamin E is consistent with reported disparities in outcomes of d-α-tocopherol studies and resolve many of the disparities of vitamin E effects on inflammation in basic and clinical research. These studies provide a basis for targeting VCAM-1-dependent signaling pathways to limit eosinophilia in disease.

This project was funded by grants from National Institutes of Health NIH R01HL069428, R01AT004837, and American Heart Association 0855583G.

INVITED SPEAKER ABSTRACTS

MECHANISMS OF EOSINOPHIL MEDIATOR SECRETION

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Human eosinophils contain within their cytoplasmic granules and secretory vesicles distinct cationic proteins, (e.g., ECP, MBP) and multiple other preformed proteins, including a diversity of cytokines. Mechanisms that govern the secretion of granule-derived proteins critically underlie the biologic activities and functions of eosinophils in health and disease.

Compound exocytosis, whereby the entire contents of granules are released extracellularly following fusion of the granule with the plasma membrane, may occur when eosinophils interact with large targets, such as helminthic parasites, but otherwise is neither commonly observed *in vivo* nor parsimonious in providing a means to selectively secrete only specific granule-derived cytokines or other proteins.

Instead, secretion of granule contents from within intact eosinophils occurs by a process termed, piecemeal degranulation (PMD). Electron microscopy, by detecting alterations in the morphology of granules within eosinophils in tissue sites, provides evidence that PMD occurs i*n vivo*. Within eosinophil granules, there is an extensive network of membranotubular structures. From this network, granule contents can be selectively mobilized into vesicles, both small round vesicles and longer curved tubular structures, that transport proteins destined for secretion at the external plasma membrane. Some proteins, such as MBP, are transported in the fluid phase of vesicles whereas others, as recognized for the cytokine, IL-4, may be transported bound to their cognate membrane-inserted receptor. Piecemeal degranulation enables differential mobilization and selective secretion of specific eosinophil granule-derived cytokines and other proteins in response to varied stimuli. Not yet known are the mechanisms that selectively mobilize specific eosinophil granule proteins into transport vesicles.

Another mechanism by which eosinophil granule-derived proteins may be specifically secreted is based on the responses of extracellular eosinophil granules. Intact, membrane-bound granules extruded from eosinophils have long been recognized in diverse disorders (e.g., asthma, dermatitis, eosinophilic Esophagitis, urticaria and helminth infections). We have demonstrated that cell-free eosinophil granules can function as independent secretory organelles capable of responding a cytokine (IFN-gamma), a chemokine (eotaxin-1), and cysteinyl leukotrienes via cognate membrane-expressed receptors, topologically oriented with ligand-binding domains displayed externally on granule membranes. The granule membrane-expressed receptors are coupled to intragranular signaling cascades and can stimulate selective, agonist-elicited secretion of ECP and cytokines from within eosinophil granules.

This project was funded by grants from the National Institutes of Health (AI020241, AI022571, AI051645) to PFW

INVITED SPEAKER ABSTRACTS

THE MAPPIT TOOLBOX: STRATEGIES TO ANALYZE MOLECULAR INTERACTIONS IN INTACT CELLS

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Background: MAPPIT (Mammalian Protein-Protein Interaction Trap) is a cytokine receptor-based two-hybrid method that operates in intact mammalian cells. Modification-independent and tyrosine phosphorylation-dependent interactions can be studied in their normal physiological context. Interactor hunts for novel protein-protein interactions can be performed using either a FACS-based protocol using complex cDNA libraries or using an array format.

Methods: Detailed technical information on the MAPPIT Toolbox can be found at www.mappit.be.

Results: Several examples will be presented on the application of MAPPIT in TLR and cytokine receptor signalling.
In addition, MAPPIT was recently used as an orthogonal assay to validate the quality of large-scale yeast two-hybrid-based protein interaction datasets, including the human interactome.

Conclusions from these studies will be discussed.

INVITED SPEAKER ABSTRACTS

EOSINOPHIL-MEDIATED IMMUNE RESPONSES TO VIRAL INFECTION

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Eosinophils have been implicated in the pathophysiology of respiratory virus infection, most typically in negative roles, such as promoting wheezing and bronchoconstriction in conjunction with virus-induced exacerbations of reactive airways disease and in association with aberrant hypersensitivity responses to viral vaccines. The vast majority of the available information on this topic focuses on respiratory syncytial virus (RSV; family Paramyxoviridae, genus *Pneumovirus*), an important pediatric pathogen that infects infants worldwide. Interestingly, although eosinophils are clearly recruited to the lung in response to formalin-inactivated RSV vaccine antigens, recent data suggest that they may not be contributing substantially to clinical pathology. Likewise, pulmonary eosinophilia is elicited in response to primary RSV infection in the youngest human infants and in neonatal mouse challenge models, yet the roles played by these virus-elicited eosinophils – negative, positive, or neutral bystander – remain to be elucidated. Finally, experiments carried out both in vitro and in vivo suggest that there are positive roles for eosinophils with respect to respiratory virus infection, as eosinophils have been shown to reduce RSV infectivity in tissue culture and promote virion clearance in RSV mouse challenge models. However, the related natural rodent pathogen, pneumonia virus of mice (PVM), is highly virulent in mice, and is not as readily cleared by eosinophils in vivo. Interestingly, PVM can replicate within eosinophils and promotes cytokine release. The molecular basis of virus replication within eosinophils and the relationship of this finding to disease outcome are currently under study.

This project was funded by NIAID Division of Intramural Research.

INVITED SPEAKER ABSTRACTS

HUMAN EOSINOPHILS EXPRESS A FUNCTIONAL γδTCR/CD3 COMPLEX: ROLE IN INNATE RESPONSES AGAINST MYCOBACTERIA AND TUMORS

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Eosinophils are effector cells during parasitic infections and allergic responses. However, their contribution to innate immunity has been only recently unravelled. The expression by human eosinophils of several receptors shared with T cells (CD4, CD25, CD28, member of the CD2 family), as well as the striking similarities between eosinophils and γδT cells (ancient lineage, mucosal preferential localisation, species specificity) led us to investigate the expression by human eosinophils of the γδTCR/CD3 complex.

In the present work, we show that human blood eosinophils express CD3 and γδ T Cell Receptor (TCR) but not αβ TCR. Surface expression of γδTCR/CD3 is heterogeneous between eosinophil donors and inducible by mycobacterial ligands. Surface immunoprecipitation revealed expression of the full γδTCR/CD3 complex. Real-time PCR amplification for CD3, γ and δ TCR constant regions transcripts showed a significantly lower expression in eosinophils than in γδT cells. Limited TCR rearrangements occur in eosinophils as shown by spectratyping analysis of CDR3 length profiles and *in situ* hybridization. Release by eosinophils of Reactive Oxygen Species, granule proteins, Eosinophil Peroxidase and Eosinophil-Derived Neurotoxin and cytokines (IFN-γ and TNF-α) was observed following activation by γδTCR-specific agonists or by mycobacteria. These effects were inhibited by anti-γδTCR blocking antibodies and antagonists. Moreover, γδTCR/CD3 was involved in eosinophil cytotoxicity against tumor cells.

Our results provide evidence that human eosinophils express a functional γδTCR/CD3 with similar, but not identical, characteristics to γδTCR from γδT cells. We propose that this receptor contributes to eosinophil innate responses against mycobacteria and tumors and may represent an additional link between lymphoid and myeloid lineages.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS : THE KILLING MACHINERY AGAINST FUNGI

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Chronic rhinosinusitis (CRS), one of the most common chronic diseases worldwide, presents histologically as an underlying, damage inflicting eosinophilic inflammation. Eosinophils are understood to play a role in the host defense against parasites, and one of the key questions was whether eosinophils play a similar role against certain airborne fungi.

After demonstrating through novel methods the presence of fungal organisms in virtually every upper respiratory track, it was found that CRS patients' immune system produces the cytokines which are crucial for the eosinophilic migration (IL-13) and activation (IL-5) when it recognizes certain common airborne molds, especially Alternaria spp. This immune response was in striking contrast to its absence in healthy controls, and independent from an IgE mediated allergy.

On CRS histology, we found that eosinophils actually migrate from the tissue into the sinus mucus and attack extramucosal fungi through the release of their toxic Major Basic Protein (MBP), which destroys the fungi but also severely damages the sinus mucosa, leading to CRS. MBP is now used for diagnostic purposes, since its presence is in the sinus lumen indicates that fungi are being attacked by eosinophils, and antifungals are used to downregulate this inflammatory response.

When multiple fungi were tested, only Alternaria spp. demonstrated an ability to induce degranulation of eosinophils. The fraction from Alternaria alternata, which induced the degranulation, had a molecular weight of ≈ 60 kDa, was highly heat labile, and worked protease dependant through a G protein-coupled receptor. Other fungal antigens, including Aspergillus, Cladosporium, and Candida, did not induce eosinophil degranulation, nor did neutrophils respond to Alternaria extracts, suggesting the presence of a fungal species and cell type specific novel innate immune response to certain fungi in human. Thus, both innate and acquired immune responses to environmental fungi, such as Alternaria (independent of IgE antibodies to Alternaria) appear to induce production of the cytokines and provide cellular activation signals necessary for the robust eosinophilic inflammation in CRS patients.

INVITED SPEAKER ABSTRACTS

VIRUS-INDUCED RELEASE OF EOSINOPHIL MEDIATORS

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Background: Virus infection is a leading cause of asthma exacerbation. Prior to exacerbation, when clinically stable, people with asthma have eosinophils in their airways. In patients with asthma exacerbation, the presence of eosinophil release products is described. Whether these eosinophils are innocent bystanders during virus infection or are actively responding to the infection is unclear.

Objectives: Generally we seek to understand how eosinophils response to respiratory viruses, in vivo and in vitro. Based on our data, we hypothesize that eosinophils do respond to virus, but through a complex interaction between antigen presenting cells and T cells.

Methods: Our *in vitro* autologous antigen-presentation system is based on the isolation of human peripheral blood monocytes, T lymphocytes and eosinophils; monocyte-derived dendritic cells (moDC) were derived from purified monocytes. We use techniques of flowcytometry and proliferation assays (BrdU incorporation chemiluminescent ELISA) to determine cell activation prior to eosinophil mediator release. We measure eosinophil release products (EPO using an OPD assay and cysteinyl-leukotrienes (Cys-LT) by ELISA). We study Parainfluenza (PIV), Respiratory Syncytial (RSV) and Rhinovirus (RV). We also quantify virus using quantitative RT-PCR, plaque assay (RSV) or hemadsorption (PIV).

Results: Viruses (PIV, RSV and RV) induce eosinophil release of EPO and CysLT. This is seen in eosinophils co-cultured with antigen presenting cells (APC), either macrophages or moDC, and T cells. It appears that CD4+ memory T cells are activated in the model as measured by CD25 and CD45RO expression. RV may have a unique ability to activate eosinophil Cys-LT release in the absence of APC. Preliminary data also suggests an antiviral property of eosinophil mediators, as we find that exposure of RSV to activated eosinophils in the presence of a bromide donor lead to the loss of 99% of viral genomic integrity.

Conclusions: Eosinophils have the ability to respond to airway viruses, and could be key immune effector cells in the response to virus infections.

This project was funded by a grant from Canadian Institutes of Health Research and AHFMR

INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND HOST DEFENSE AGAINST BACTERIA

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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. The aim of this study was to better understand the role of eosinophils within the gastrointestinal immune system. We show here that lipopolysaccharide (LPS) from gram-negative bacteria activates interleukin (IL)-5 or interferon (IFN)-γ primed eosinophils to release mitochondrial DNA in a reactive oxygen species (ROS) dependent manner, but independent of eosinophil death. 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 the granule proteins form extracellular structures able to bind and kill bacteria both in vitro and under inflammatory conditions in vivo. Moreover, following cecal ligation and puncture, IL-5 transgenic but not wild-type mice demonstrated intestinal eosinophil infiltration and extracellular DNA deposition in association with protection against microbial sepsis. These data suggest a novel mechanism of eosinophil-mediated innate immune responses that might be important to maintain the intestinal barrier function following inflammation-associated epithelial cell damage preventing the host from uncontrolled invasion of bacteria.

INVITED SPEAKER ABSTRACTS

BACTERIA AND EOSINOPHILIC ACTIVATION

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Background: Neutrophilic granulocytes are the primary cellular defense against invasive bacterial infections in humans. Eosinophilic granulocytes are also endowed with bactericidal activity, even though their main antimicrobial function is to protect us against the deleterious effects of infestation with helminthic parasites.

Objectives: To elucidate if eosinophilic granulocytes are able to inactivate all types of bacteria, or if they show specific activity against defined bacterial species. The types of eosinophilic activation patterns evoked by bacterial exposure were determined. The role of formyl peptide receptors in mediating bacterial activation of eosinophils was also investigated.

Methods: Eosinophils derived from fresh buffy coats were exposed to 11 different bacterial species, representative of various branches of the bacterial phylogenetic tree. The ability of the eosinophils to evoke chemotaxis, respiratory burst, degranulation and release of cytokines was studied. The ability of antagonists of the formyl peptide receptor family to block bacterial interaction with eosinophils was assessed.

Results: Both grampositive and gramnegative bacteria induced chemotaxis and respiratory burst in human eosinophils. Gramnegatives tended to cause higher release of ECP in eosinophils than grampositives, both types of bacteria gave rise to degranulation of EPO and MBP. Occasional secretion of IL-13 was evoked by bacterial species. Whereas certain bacterial species triggered all the investigated forms of eosinophilic activation, others did not evoke any reactivity in eosinophils whatsoever. Pretreatment of eosinophils with the formyl peptide receptor antagonist Cyclosporin H caused a dramatic reduction in respiratory burst triggered by bacteria. However, the magnitude and length of the respiratory burst evoked by bacteria and the prototype ligand of the formyl peptide receptor, the tripeptide fMLF, were different. This indicates that other substances besides fMLF mediated bacterial-induced release of oxygen radicals in eosinophils.

Conclusions: Select bacterial species can elicit eosinophilic activation. Since grampositive and gramnegative bacteria had this ability in common, LPS is not likely to be the major bacterial mediator. Bacterial activation of human eosinophils occurs partly via formyl peptide receptors and/or signal transduction pathways distal to this receptor family.

This project was funded by grants from the Swedish Research Council, Cancer and Allergy Foundation, ALF Funding, and the Swedish Allergy and Asthma Foundation.

INVITED SPEAKER ABSTRACTS

NEWLY DISCOVERED ROLES FOR BASOPHILS: A NEGLECTED MINORITY GAINS NEW RESPECT

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Basophils represent less than 1% of peripheral blood leukocytes. Because of this smallest size of population and their similarity to mast cells, including basophilic granules in the cytoplasm, surface expression of high affinity IgE receptor and histamine release, basophils have often been neglected or considered to be minor and redundant 'circulating mast cells'. Basophils are often recruited to the site of allergic inflammation, albeit in small numbers, but it remained uncertain whether basophils play any significant role in allergic reactions. We have recently demonstrated that basophils play critical roles in systemic anaphylaxis and chronic allergic inflammation, distinctly from mast cells.

Basophils are one of the major players in the IgG- but not IgE-mediated systemic anaphylaxis, in contrast to mast cells. In response to the allergen-IgG immune complexes, basophils release the platelet-activating factor rather than histamine as the major chemical mediator to induce the systemic anaphylaxis. The depletion of basophils protects mice from death due to anaphylactic shock. Basophils also play a crucial role in the development of the IgE-mediated chronic allergic inflammation (IgE-CAI) with massive eosinophil infiltration in the skin, independently of T cells and mast cells, even though basophils account for only ~2% of the infiltrates. The basophil depletion shows a therapeutic effect on on-going allergic inflammation. Accumulating evidence suggests that basophils function as initiators rather than effectors of the chronic allergic inflammation. Unexpectedly, we also found that at the later phase of IgE-CAI, basophils play an important role in the resolution of allergic inflammation. Antigen/IgE-activated basophils induce alternative activation of macrophages through Th2 cytokine release, and the basophil-elicited, alternatively-activated macrophages in turn induce apoptosis of neutrophils, leading to the resolution of inflammation. The depletion or developmental block of alternatively-activated macrophages resulted in accelerated inflammation with a massive accumulation of neutrophils. Thus, basophils, in spite of their small number, have fundamental and non-redundant roles in IgE-CAI, not only as initiators but also as terminators of inflammation, via distinct mechanisms.

Basophils are no longer regarded as redundant because of their similarities to mast cells. Basophils and their products seem to be promising therapeutic targets for allergic disorders.

This project was funded by Grants-in-Aid from the Japanese Ministry of Education, culture, Sports, Science and Technology.

INVITED SPEAKER ABSTRACTS

NEUTROPHILS AND ECP

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The production of eosinophil cationic protein (ECP) in IgE-dependent diseases has been associated mainly with eosinophils, although no IgE-dependent ECP release has been observed in these cells. Since there is increasing evidence of neutrophil participation in allergic processes, we have examined whether human neutrophils are able to produce ECP by an IgE-dependent mechanism. Different allergens were evaluated, and we also challenged the neutrophils with mAb to FcεRI, FcεRII, and galectin-3 receptors In this work we show, that ECP is produced by human neutrophils from allergic patients, after challenge with allergen, anti-IgE antibody, and platelet-activating factor (PAF). The challenge with antibodies against FcεRI and galectin-3 released ECP in both cases (FcεRI>galectin-3). Challenge with antibody against FCεRII did not induce release of ECP. No ECP mRNA was detected in unstimulated cells, but message was present when cells were stimulated by an IgE-dependent mechanism. Expression at both mRNA and protein levels was induced by an IgE-dependent mechanism. These observations represent a novel view of neutrophils as possible source of ECP in the IgE-dependent diseases.

This project was funded by a grant from the Fundación Alergol, Fundación SEAIC of Spain.

INVITED SPEAKER ABSTRACTS

ROLE OF BASOPHILS IN IMMUNOLOGICAL MEMORY RESPONSES

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Basophils have recently been recognized as important contributors to cellular and humoral memory immune responses. Basophils and antigen-specific B cells are the only leukocyte populations that can bind significant amounts of intact antigen on their cell surface during a memory immune response in vivo. High affinity binding of antigen to basophils occurs via capture of antigen specific immunoglobulins of the IgE isotype. Low affinity binding and activation of basophils is also mediated by IgG antibodies and low affinity Fc-receptors. Several additional ways to activate basophils will be discussed. During a memory immune response basophils are significantly redistributed throughout lymphoid tissues and are the most important source of IL-4 and IL-6 in the lymphoid tissues. Thereby basophils markedly alter the phenotype of CD4+ T cells and support B cell responses. The impact of basophils will be demonstrated in models of immunization with protein antigens, in vaccination and infection experiments with streptococcus pneumoniae and in the model of collagen induced arthritis.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS IN HEALTH AND DISEASE: THE *LIAR* HYPOTHESIS

James J. Lee

I suspect that the title of this presentation will be surprising/confusing to anyone who graduated from medical school. After all, during year two everyone took Hematology (using a frighteningly large textbook entitled "Hematology: Basic Principles and Practice" edited by Hoffman, Benz, Shattil, Furie and Cohen) and were invariably taught: Eosinophils are end-stage effector cells with destructive capabilities mediated predominantly by the release of cytotoxic cationic granule proteins. Moreover, this explanation was then further confirmed by the fact that the word eosinophil in the medical literature invariably is contained in a sentence which either precedes or follows a sentence with the word helminth or possibly asthma. This has led to an almost fatalist view of eosinophils and the strategies with which to treat patients that would make even William of Ockham proud - eosinophil effector functions are bad and "the only good eosinophils are dead eosinophils". Unfortunately, the strengths of any dogma are also its greatest weaknesses. Namely, while the simplicity of dogmatic concepts and the repetitive proclamation by authority as to their truths build consensus and lowers the entropy surrounding difficult issues and decisions, they often ignore details (i.e., data not easily explained by the dogma) and are intolerant of alternative "out-of-the-box" hypotheses.

The goal of my presentation is two fold: (i) I will review recent salient observations regarding eosinophils and their activities as well as reinterpret earlier data as part of the synthesis of a new paradigm describing eosinophils as mediators of Local Immunity And/or Remodeling in both health and disease - The LIAR Hypothesis; (ii) I hope to be inflammatory (pun intended!) and suggest that the resulting discussions and debate will be thought-provoking and, more importantly, lead to a greater understanding of how eosinophils are both important for the maintenance of homeostatic baseline and the exacerbation/suppression of disease symptoms.

INVITED SPEAKER ABSTRACTS

TH2, TH17, AND ALLERGIC INFLAMMATION

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Background: IL-23-IL-17 producing $CD4^+$ T cell (Th17 cell) axis plays a crucial role in the development of chronic inflammatory diseases including autoimmune diseases. However, the role of IL-23-Th17 cell axis in the regulation of allergic airway inflammation is still largely unknown.

Objectives: To determine the role of IL-23-Th17 cell axis in allergic airway inflammation.

Methods: We examined the effect of anti-IL-23 p19 antibody on antigen-induced airway inflammation. We also investigated the effect of enforced expression of IL-23 on allergic airway inflammation by generating lung-specific IL-23 transgenic mice. Moreover, we examined the effect of adoptive transfer of antigen-specific Th17 cells on allergic airway inflammation.

Results: IL-23 p19 and p40 but not IL-12 p35 were expressed in the lung of sensitized mice upon antigen inhalation and the neutralization of IL-23 p19 decreased antigen-induced eosinophil recruitment and Th2 cytokine production in the airways. The enforced expression of IL-23 in the airways significantly enhanced antigen-induced eosinophil and neutrophil recruitment into the airways, IL-13, IL-17A, and TNF-a production in the airways, goblet cell hyperplasia, and airway hyperresponsiveness. Moreover, IL-23-mediated enhancement of antigen-induced Th2 cytokine production and eosinophil recruitment into the airways were still observed in mice lacking IL-17A. Furthermore, although adoptive transfer of antigen-specific Th17 cells alone induced neutrophil but not eosinophil recruitment into the airways upon antigen inhalation, co-transfer of Th17 cells with Th2 cells significantly enhanced antigen-induced, Th2 cell-mediated eosinophil recruitment into the airways and airway hyperresponsiveness.

Conclusions: IL-23-Th17 cell axis not only induces Th17 cell-mediated neutrophilic airway inflammation but also enhances Th2 cell-mediated eosinophilic airway inflammation.

This project was funded by Grants-in-Aids for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government and by Global COE Program (Global Center for Education and Research in Immune System Regulation and Treatment), MEXT, Japan.

INVITED SPEAKER ABSTRACTS

MICRORNA REGULATION OF T_H2 MEDIATED ALLERGIC AIRWAYS DISEASE

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Allergic asthma is an inflammatory disease of the lung characterised by abnormal T helper-2 (T_H2) lymphocyte responses to inhaled antigens. The molecular mechanisms leading to the generation of T_H2 responses remain unclear, although toll-like receptors (TLRs) present on innate immune cells play a pivotal role in sensing molecular patterns and in programming adaptive T cell responses. Here we show that *in vivo* activation of TLR4 by house dust mite antigens leads to the induction of allergic disease, a process that is associated with expression of a unique subset of small, non-coding microRNAs. Selective blockade of microRNA (miR) suppressed the asthmatic phenotype, resulting in diminished T_H2 responses, inflammation, airways hyperresponsiveness, eosinophil recruitment and mucus hypersecretion. MiR blockade resulted in augmented expression of factors alters T_H2 cell function via negative regulation of GATA3 expression. In summary, this study a functional connection between miRNA expression and asthma pathogenesis is demonstrated, and our data suggest that targeting miRNA in the airways may lead to new anti-inflammatory treatments for allergic asthma.

INVITED SPEAKER ABSTRACTS

REGULATION OF ALLERGIC INFLAMMATION AND AHR BY T CELL SUBSETS

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In order to try and directly regulate allergen driven Th2 responses in the lung we used a mouse model of airways inflammation to examine the suppressive activity of allergen-specific $CD4^+CD25^+$ T cells in vivo. Transfer of ovalbumin-peptide-specific $CD4^+CD25^+$ T cells to ovalbumin-sensitised mice reduced airway hyperreactivity (AHR), recruitment of eosinophils, and Th2 cytokine expression in the lung following allergen challenge. This suppression was dependent on IL-10 since increased lung expression of IL-10 was detected after transfer of $CD4^+CD25^+$ T cells and regulation was reversed by anti-IL-10R antibody. However, suppression of AHR, airway inflammation, and increased expression of IL-10 were still observed when $CD4^+CD25^+$ T cells from IL-10 gene deficient mice were transferred. Intracellular cytokine staining confirmed that transfer of $CD4^+CD25^+$ T cells induced IL-10 expression in recipient $CD4^+$ T cells, but no increase in IL-10 expression was detected in airway macrophages, dendritic cells or B cells. These data suggest that $CD4^+CD25^+$ T cells can suppress the Th2 cell-driven response to allergen *in vivo* by an IL-10 dependent mechanism, but that IL-10 production by the regulatory T cells themselves is not required for such suppression. Therefore, strategies designed to increase the number of IL-10 secreting cells may be of benefit for treatment of allergic inflammation.

Treatment of patients with allergic asthma using low doses of peptidic T cell epitopes reduces allergic sensitization and improves surrogate markers of disease. We have developed a novel HLA-DR1 transgenic murine model of asthma in order to investigate the molecular mechanism behind this regulation. Tracking of allergen specific T cells using DR1 tetramers determined that suppression was associated with the induction of $IL\text{-}10^+$ T cells that were more abundant than T cells specific for the treatment peptide. Moreover, resolution of airway pathophysiology in this model was associated with reduced recruitment, proliferation and effector function of allergen specific Th2 cells. Our results provide evidence of linked epitope suppression *in vivo* and IL-10 induction in a HLA-DR1 transgenic mouse model designed to closely mimic peptide therapy in humans.

Our data suggest that IL-10 secreting regulatory cells, either transferred or induced *in vivo* are able to down-modulate allergen-induced airway inflammation and hyperreactivity.

INVITED SPEAKER ABSTRACTS

TSLP, OX40L AND IL-25 IN ALLERGIC IMMUNE RESPONSES

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Allergic diseases are often triggered by environmental allergens that induce dominant type2 immune responses, characterized by the infiltrated T_H2 lymphocytes, eosinophils, and elevated T_H2 cytokines. In addition to T_H2 type immune responses, epithelial stress and injury linked to tissue remodelling are often observed, suggesting that epithelial cells may play important role in regulating allergic responses. Dendritic cells (DCs), the professional antigen-presenting cells with the capabilities of sampling allergens, are considered as the key player on instructing T_H2 immune responses. Whether inflamed epithelium can regulate innate immunity, such as macrophages and DCs, which in turns instruct adaptive immunity has long been hypothesized. Studies of TSLP (thymic stromal lymphopoietin), an epithelial cells-derived cytokine, that can strongly activate DCs, provide important evidences that the epithelial barrier can trigger allergic diseases by regulating immune responses. The finding that OX40/OX40L interactions are the molecular trigger responsible for the induction and maintenance of T_H2 responses by TSLP-activated DCs provides a plausible molecular explanation for TSLP-mediated allergy. Recent progresses in characterizing the proinflammatory IL-17 cytokine family have added an additional layer of complexity on the regulation of allergic inflammation. TSLP-DCs can induce a robust expansion of T_H2 memory cells and strengthen functional attributes by upregulating their surface expression of IL-17RB (IL-25R), the receptor for cytokine IL-17E (IL-25), a distinct member of IL-17 cytokine family. IL-17E (also know as IL-25) produced by epithelial cells, and other innate cells, such as eosinphils, basophils, and mast cells, are shown to regulate adaptive immunity by enhancing T_H2 cytokine productions. The reciprocal expression of IL-25 and its cognate receptor IL-25R by eosinophils/basophils and activated $CRTH2^+T_H2$ memory cells, respectively suggests that the IL-25-mediated cross talk between eosinophils/basophils and T_H2 memory cells may occur during chronic lung inflammation resulting in the enhanced airway allergy driven by the amplified T_H2 immune response. These exciting findings expand our knowledge of the complex immunological cascades that result in allergic inflammation and may provide novel therapeutic approaches for the treatments of allergic diseases.

This work is supported by grant NIH- A1061645-01.

INVITED SPEAKER ABSTRACTS

INVOLVEMENT OF CRTH2 IN EOSINOPHILIC INFLAMMATION - BASIC BIOLOGY AND CLINICAL FINDINGS

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Chemoattractant receptor-homologous receptor expressed on Th2 cells (CRTH2, also known as DP_2) is selectively expressed by Th2 cells, eosinophils and basophils and mediates activation of these cells in response to the mast cell product prostaglandin D_2 (Hirai et al, 2001). There is increasing evidence that CRTH2 plays a central role in key aspects of the allergic response. Activation of CRTH2 on Th2 cells leads to the elaboration of cytokines such as IL-4, IL-5 and IL-13 in absence T cell receptor activation or any form of co-stimulation (Xue et al, 2005). These cytokines have downstream effects on the production of IgE and in promoting tissue eosinophilia. The ability of mast cells to activate both Th2 cells and eosinophils *in vitro* is mediated by CRTH2 (Gyles et al, 2006; Xue et al, 2009). These findings extend to various *in vivo* models of allergic inflammation where the use of small molecule CRTH2 antagonists or mice genetic deficient in CRTH2 is associated with reduced production of Th2 cytokines, lower levels of circulating IgE and diminished airway eosinophilia. The dominant role played by CRTH2 in mediating interactions between cells involved in the allergic response has spurred interest in the discovery of antagonists of this receptor for the treatment of asthma and related disorders. OC000459 is a highly potent, selective and orally active CRTH2 antagonist which has recently completed Phase IIa proof-of-concept trials in asthma and allergic rhinitis. The outcome from these clinical trials will be presented providing preliminary evidence that OC000459 is effective in reducing airway inflammation (as measured by sputum eosinophilia) and this effect is associated with an improvement in lung function and asthma symptoms.

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INVITED SPEAKER ABSTRACTS

ROLES OF THE EPITHELIAL 'ALARMIN', IL-33, IN EOSINOPHILIC INFLAMMATION

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During the past 10 years, considerable progress has been made towards a better understanding for how the immune system senses foreign pathogens and tissue damage. Cells that express members of the interleukin (IL)-1/Toll-like receptor (IL-1R/TLR) family on their surfaces respond to pathogen-associated molecular patterns. The immune system can also be activated by endogenous danger signals provided by stressed or necrotic cells. Some investigators have proposed the term 'alarmin' to characterize the proteins that are rapidly released from cells in response to infection or tissue damage.

IL-33 is a member of the IL-1 family of cytokines and is found in the nucleus of tissue cells, such as endothelial cells and epithelial cells. We found that IL-33 is constitutively expressed by normal human bronchial epithelial cells (NHBE) and is localized to the nucleus. The turnover of IL-33 is relatively quick, and when NHBE cells were treated with actinomycin D for 6 hours, they became depleted of IL-33. IL-33 was released by NHBE cells when they were exposed to pharmacological and natural agents that induce an increase in intracellular calcium concentrations, such as ionomycin and an extract from the fungus *Alternaria alternata*. Cellular damage that was induced by exposure to freeze-thaw cycles or detergents also induced IL-33 release from NHBE cells.

We found that dendritic cells (DCs) express the necessary receptor for IL-33 (ST2), and they respond to IL-33 by producing IL-6 and expressing co-stimulatory molecules. IL-33, when cultured together with DCs and naïve CD4+ T cells, has the ability to initiate a Th2-type response characterized by marked production of IL-5 and IL-13 in vitro. Similarly, lysates of NHBE cells were able to induce the Th2-type cytokine response in a mixed culture of DCs and naïve CD4+ T cells; this response can be blocked by anti-ST2 antibody.

When administered to the airways of naïve mice, IL-33 induced robust production of IL-5 and IL-13 from a novel immune cell(s) in the lungs with unique phenotypic characteristics as follows: B220-, CD3-, CD4-, CD8-, CD14-, CD16/32-, DX5-, TCRγ/δ-, c-Kit-, FcεRIa-, MHCII+/-, CD25+, and CD44+. Furthermore, airway administration of IL-33 together with exogenous model antigens, such as OVA and KLH, induced production of IL-4 in naïve CD4+ T cells in lung draining lymph nodes. In response to these model antigens, IL-33 also induced Th2-type memory CD4+ T cells and humoral immune responses (e.g. IgE and IgG1 production) in an IL-4-dependent manner in vivo.

Thus, IL-33 is likely released by damaged or stressed airway epithelial cells and has the capacity to induce both innate and adaptive Th2-type immune responses in vitro and in vivo. Further studies are necessary to investigate the role(s) of IL-33 in the development of Th2-type immune responses in mucosal organs and in the pathophysiology of eosinophilic disorders in humans.

Supported by grants from the National Institutes of Health, AI34486 and AI71106, and by the Mayo Foundation.

INVITED SPEAKER ABSTRACTS

INVOLVEMENT OF IL-9 IN ALLERGY AND PARASITOSIS

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Increased IL-9 expression favors intestinal helminth parasite expulsion and induces an asthma-like phenotype in lungs, including mucus overproduction, mastocytosis, lung eosinophilia, airway hyperresponsiveness, correlating with increased production of other Th2 cytokines such as IL-4, IL-5 and IL-13. In order to determine the exact role of IL-13 in this phenotype, as IL-13 overexpressing mice develop the same asthmatic profil, mice overexpressing IL-9 were crossed with IL-13-deficient mice. In these animals, IL-9 could still induce mastocytosis and B lymphocyte infiltration of the lungs. While IL-9-induced eosinophilia in the peritoneal cavity was not diminished in absence of IL-13, IL-13 was required for IL-9 to increase eosinophil chemoattractant expression, and therefore, lung eosinophilia. However, mucus production and upregulation of lung epithelial genes upon IL-9 overexpression were completely abolished in absence of IL-13. Additional experiments of hematopoietic cells transfer into IL-9R -/- mice indicated that IL-13 was a direct mediator, and not a cofactor, of IL-9 effects on lung epithelial cells.

In the same line of observations, in colon from IL-9 transgenic mice, intestinal mucin gene together with goblet cells-associated genes were upregulated in an IL-13 dependent way. Additionnally, typical Paneth cell markers were found to have an enhance expression in response to IL-9. Histochemical staining of Paneth cells by phloxine/tartrazine confirmed that IL-9 induced Paneth cells hyperplasia in Lieberkühn glands of the small intestine, and in the colonic mucosa, where this cell type is normally absent. The upregulation of these Paneth cell-specific genes by IL-9 required IL-13 like for the other epithelial effects of IL-9.

Taken together, these data indicate that IL-9 can promote allergy and anti-parasite responses through IL-13-independent pathways via mast cells, eosinophils and B cells, and through IL-13 induction by hematopoietic cells for mucus production by epithelial cells and for Paneth cell hyperplasia.

INVITED SPEAKER ABSTRACTS

EOXINS: NOVEL PROINFLAMMATORY ARACHIDONIC ACID METABOLITES PRODUCED BY HUMAN EOSINOPHILS

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Human eosinophils contain abundant amounts of 15-lipoxygenase-1 (15-LO-1). The biological role of 15-LO-1 in humans is, however, unclear. Studies on the metabolism of arachidonic acid in human eosinophils led to the discovery of a novel group of mediators, formed via the 15-LO-1 pathway. Since eosinophils are such an abundant source of these metabolites, we called these mediators eoxins. The enzyme 15-LO-1 catalyses the conversion of arachidonic acid to eoxin (EX) A_4, which in turn can be conjugated with glutathione, leading to the formation of EXC_4. This metabolite can be further metabolized to EXD_4 and EXE_4. Eosinophils produced EXC_4 after challenge with the proinflammatory mediators leukotriene (LT) C_4, prostaglandin D_2 and interleukin-5, showing that EXC_4 can be synthesized from the endogenous pool of arachidonic acid. Eoxins induced increased permeability of endothelial cell monolayer in vitro, indicating that eoxins can modulate and enhance vascular permeability, a hallmark of inflammation. In this model system, eoxins were about 100 times more potent than histamine and almost as potent as LTC_4 and LTD_4. Studies on the metabolism of arachidonic acid in eosinophils isolated from patients with different variant of asthma showed that eosinophils from patients with severe asthma (SA) or aspirin-intolerant asthma (AIA) had a markedly higher 15-LO-1 activity than eosinophils from healthy volunteers or mild asthmatics. The total levels of EXC_4 and LTC_4 were not significantly higher in any investigated group but the formation of EXC_4 and LTC_4 increased in eosinophils isolated from SA and AIA patients if the cells were pretreated with aspirin prior to activation. These findings may have important diagnostic and therapeutic implications.

INVITED SPEAKER ABSTRACTS

MECHANISTIC DISSECTION OF EOSINOPHILIC GASTROINTESTINAL DISORDERS (EGID)

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Eosinophilic esophagitis, a clinical disorder recently recognized and growing over the past decade, has the unique features of chronic esophagitis, atopy, immune sensitization to oral antigens, reversibility and familial association. Symptoms frequently mimic those of gastroesophageal reflux disease (GERD) but the two diseases are distinct in terms of their histopathology, genetic signature, response to therapy, hereditary risk and association with allergy. Transcript signatures and animal models have uncovered the importance of adaptive T cell immunity and IL-13 elicited esophageal epithelial cell responses and the eosinophil chemoattractant eotaxin-3. Notably, symptoms, dysregulated esophageal gene expression and pathology are largely reversible following reduction of specific food antigen exposure, as well as anti-inflammatory therapy, but chronic treatment is necessary to prevent relapse. The molecular pathways, primary genetic events, and their interplay with environmental triggers will be reviewed.

This project was funded by grants from NIH-NAID, NIH-NIDDK, Dana Foundation, CURED Foundation, Food Allergy Project, and Cincinnati Children's Hospital Research Foundation

INVITED SPEAKER ABSTRACTS

EOSINOPHILIC ESOPHAGITIS: AN OMNIUM GATHERUM, A VARIANT OF GERD, OR A SPECIFIC DISEASE?

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Eosinophilic Esophagitis (EoE) is a rapidly emerging, chronic-inflammatory disease of the esophagus.
The first articles reporting patients with dysphagia and an impressive eosinophilic inflammation of the esophagus – meanwhile recognized as EoE – were published more than three decades ago. Unaware of this particular entity, the cases were (mis)-interpreted as achalasia, fungal infections, variants of gastro-esophageal reflux disease (GERD) or as eosinophilic gastroenteritis. The eosinophilic infiltration was regarded as a non-specific epi-phenomenon occurring under a variety of conditions, and *EoE was therefore considered as an omnium gatherum*.

Fifteen years later three seminal case series were published providing a comprehensive description of the clinical and pathological features of EoE, and the disease was recognized as an own standing entity. Immunologist and gastroenterologist begun to focus on this disease and in 2007 a panel of international EoE-experts (TIGERS) summarized the clinical, histo-pathologic, endoscopic, immuno-pathogenical and epidemiological features of EoE in a consensus paper and developed the following diagnostic criteria: “Eosinophilic esophagitis (EoE) is characterized by esophageal symptoms and a dense tissue eosinophilia, both refractory to proton pump inhibitors”. This description takes into account the complexity of the diagnosis containing clinical and pathological features, as well as the distinction from GERD. Basing on this consensus paper, *EoE was regarded as a well defined entity.*

Today, EoE is diagnosed regularly in GE- and Allergy-Practices and specialized centres take care of hundreds of EoE patients. This rapidly increased experience has opened a new view of EoE: Besides the classical form – affecting mainly male individuals with an atopic background, involving the superficial mucosa layers of the esophagus and responding rapidly to treatment with topical corticosteroids – several variant forms of EoE have been recognized. A first variant covers all clinical, endoscopical and histological features of to the classical form mentioned below, but responds perfectly to treatment with PPI. In the absence of signs of GERD, this variant is denominated “PPI-responsive EoE”. Another variant of EoE is characterized by involving mainly the deeper layers of the esophageal wall and seems to affect mainly older patients. This form leads to atypical chest pain and segmental stenosis of the esophagus. The diagnosis of this subform is hampered by the fact that the mucosa is minimally inflamed and endoscopic biopsies may therefore fail to establish the diagnosis. In addition, a broad range of individual disease courses are observed, from mild or even subclinical forms to aggressive types leading to severe remodeling and dysfunction of the esophagus. Basing on this growing clinical experience, there is good evidence that there is not one single but rather several sub-forms of EoE. *EoE is therefore again an omnium gatherum* with the need for further investigation, in order to improve the diagnostic and therapeutic approaches.

INVITED SPEAKER ABSTRACTS

NOVEL MECHANISMS OF EOSINOPHIL GRANULE PROTEIN-INDUCED SKIN LESION FORMATION

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Background: Eosinophil granule proteins are deposited in cutaneous lesions in many human diseases, but how these proteins contribute to disease is obscure.

Objectives: To determine mechanisms of eosinophil involvement in skin diseases.

Methods: We injected eosinophil cationic protein (ECP or RNase 3), eosinophil-derived neurotoxin (EDN or RNase 2), eosinophil peroxidase (EPO), and major basic protein-1 (MBP1) intradermally into guinea pig and rabbit skin. We modified biochemical activities of the granule proteins to determine their effects on the tissues.

Results: ECP and EDN each induced distinct skin lesions at ≥ 2.5 μM that began at 2 days, peaking about 7 days and persisting up to 6 weeks. These lesions were ulcerated (ECP) or crusted (EDN) with marked cellular infiltration. EPO and MBP1, 10 μM, each produced perceptible induration and erythema with moderate cellular infiltration resolving within 2 weeks. ECP and EDN localized to dermal cells within 2 days whereas EPO and MBP1 remained extracellular. Overall, cellular localization and RNase activity of ECP and EDN were critical for lesion formation; differential glycosylation, net cationic charge, or RNase activity alone did not account for lesion formation. Ulcerated lesions from patients with the hypereosinophilic syndrome showed ECP and EDN deposition comparable to that in guinea pig skin.

Conclusions: ECP and EDN disrupt skin integrity and cause inflammation. Their presence in ulcerative skin lesions may explain certain findings in human eosinophil-associated diseases.

This project was funded by grants from: National Institutes of Health Grants AI 11483, AI 09728, AI07047 Training Grant, AI 34577, AI 50494; American Academy of Allergy, Asthma and Immunology Women Physicians in Allergy Grant (KML); The Mayo Foundation, the Kieckhefer Foundation, and the Dr. Smith H. and Lucille Gibson Postdoctoral Research Fellowship in Dermatology (DAP).

INVITED SPEAKER ABSTRACTS

CORD BLOOD EOSINOPHIL PROGENITOR PHENOTYPES AND RISKS FOR DEVELOPMENT OF ATOPY AND ASTHMA IN EARLY LIFE

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There is now a burgeoning body of evidence showing that activation of selective hemopoietic processes is not only associated with the onset and maintenance of allergic inflammation in atopic adults, but also with the *development* of the allergic diathesis in infants. This area promises to be of great interest in understanding the role and fate of the very abundant $CD34^+$ stem cell (SC) populations present in cord blood (CB). Dysfunctional *adaptive* (T-cell) immunity in the genesis of atopy and asthma is paralleled by abnormalities in *innate* immunity, including the contribution of SC, particularly progenitors of the eosinophil-basophil (Eo/B) lineage. Very recent observations we have made suggest a predominant role for Thymic Stromal Lymphopoietin (TSLP) in induction of TH2 cytokines in bone marrow, sputum and CB $CD34^+$ cells; these findings provide a potential link between adaptive and innate immune responses in orchestrating both tissue-specific and systemic Eo/B differentiation and thus allergic inflammation.

In several birth cohort studies, we have found that CB Eo/B progenitor hemopoietic cytokine receptors are associated with increased atopic risk, showing an *inverse* correlation between maternal skin prick test responses to common allergens and IL-3Rα, IL-5Rα, and GM-CSFRα expression on CB $CD34^+$ cells at birth. These alterations in CB progenitors also correlate with clinical outcomes at one year, predicting both *atopic dermatitis* and *wheeze*, the latter in response to acute respiratory illness (ARI) in the first year; CB Eo/B progenitors can be modified by maternal dietary intervention during pregnancy. These results provide insights into novel mechanisms for predicting the generation of tissue airway eosinophilic inflammation in infancy and early childhood, in response to viral or allergenic stimuli.

Real-time polymerase chain reaction (Q-PCR) has recently been employed in our laboratory to ascertain the kinetic patterns of expression of CB Eo/B-lineage specific genes, GATA-1, MBP and IL-5Rα. Stimulation with IL-5 results in an early up-regulation of GATA-1 expression, peaking at 24-48h. In contrast, MBP is up-regulated in a slowly progressive pattern, maximally at 72h, while there is stable, low expression of IL-5Rα, with differential expression of mRNA for IL-5R soluble and transmembrane isoforms. Numbers of Eo/B-CFU relate to antecedent GATA-1, and inversely to MBP, expression. Molecular markers of critical differentiation-specific events in CB SC may herald future atopic and asthmatic biological and clinical outcomes.

Finally, since atopy in early life may be modulated by the expression and stimulation of Toll-like receptors (TLR) on immunocompetent cells, we also have investigated the expression of TLR on CB progenitors, demonstrating significant TLR-2, -4, and -9 expression; ligation of TLR-4 with lipopolysaccharide decreases percentage co-expression and increases mean fluorescence intensity of IL-3Rα, IL-5Rα and GM-CSFRα on CB $CD34^+$ cells. These alterations may have functional consequences for CB progenitor Eo-lineage differentiation, indicating an alternate innate immune pathway of microbial influence on development of allergic inflammation and atopy in early life.

This project was funded by grants from the Canadian Institutes for Health Research and AllerGen NCE Inc.

INVITED SPEAKER ABSTRACTS

CONTRIBUTION OF EOSINOPHILS TO RSV VACCINE ENHANCED DISEASE

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Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract disease in children. Children previously vaccinated with a formalin-inactivated (FI) RSV vaccine experienced enhanced morbidity and mortality upon natural RSV infection. Histological analysis revealed the presence of eosinophils in the pulmonary infiltrate of the vaccinated children. Immunization of BALB/c mice with FI-RSV or a recombinant vaccinia virus (vacv) expressing the RSV attachment (G) protein results in pulmonary eosinophilia and increased pulmonary disease after RSV challenge that closely mimics the RSV vaccine-enhanced disease observed in humans. The underlying causes of RSV vaccine-enhanced disease remain poorly understood. We show that RSV infection of eosinophil-deficient mice previously immunized with vacvG results in the development of increased weight loss, clinical illness, and airway obstruction that are similar to wild-type controls. Surprisingly, we demonstrate that these measures of RSV vaccine-enhanced disease are not associated with Th2 responses but are instead dependent upon STAT4. Interestingly, neither IL-12 nor IL-23, the two most common STAT4-activating cytokines, proved necessary for the development of disease. We demonstrate that IFN-γ, which is produced following STAT4 activation, contributes to clinical illness and increased airway obstruction, but not weight loss. Our results have important implications for future RSV vaccine design suggesting that enhancing a Th1 response may exacerbate disease.

This project was funded by a grant from the NIH.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND ANGIOGENESIS – UPDATE ON MECHANISMS INVOLVED IN EOSINOPHIL-ASSOCIATED TISSUE REMODELING

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Background: Eosinophils (EOS) are critically involved in allergic reactions where both inflammation and tissue remodeling are present with angiogenesis and tissue hypoxia. We have recently demonstrated that EOS can actively contribute to angiogenesis in part by their production of VEGF. EOS derived MBP is known to be an active player in all the stages of allergic inflammation. Moreover we have found that osteopontin (OPN) exhibits pro-fibrogenic properties in a murine model of chronic asthma and is expressed in lung infiltrated EOS.

Objectives: To further investigate EOS contribution to angiogenesis by evaluating the pro-angiogenic properties of MBP and the expression and function of OPN in human EOS.

Methods: Rat aortic endothelial cells and human umbilical vascular endothelial cells were cultured with MBP and their viability (Trypan blue), proliferation (Thymidine) and capillary-like structure formation (matrigel) were investigated. The angiogenic activity of MBP was also tested in vivo using the chick chorio allantoic membrane (CAM) assay.
OPN mRNA (RT-PCR) and protein (Immunoflourescence) expression in peripheral blood EOS from atopic subjects were evaluated. OPN/release was determined in resting and activated (GM-CSF, IL-5) EOS. The contribution of OPN to EOS-induced angiogenesis was determined using CAM assay and OPN-induced EOS chemotaxis was determined (ChemoTx System). Finally, OPN expression in BALF from mild asthmatic and normal control subjects was determined.

Results: Subcytotoxic concentrations of MBP induced endothelial cell proliferatioin and enhanced the pro-mitogenic effect of VEGF, but did not affect their VEGF release. MBP promoted capillarogenesis by endothelial cells seeded on matrigel and sprouting formation in the CAM assay. Furthermore, the pro-angiogenic effect of MBP was not due to its cationic charge since stimulation of the CAMs with the synthetic polycation, poly-L-arginine does not induce any angiogenic effect.
OPN was found to be expressed in human EOS and it was increased following GM-CSF and IL-5 activation. EOS-derived OPN contributed to EOS-induced angiogenesis in the CAM assay. Moreover OPN promoted eosinophil chemotaxis and this effect was shown to be mediated by $\alpha_4\beta_1$ integrin binding. OPN is increased in the bronchoalveolar lavage fluid from mild asthmatic subjects and correlates with EOS counts.

Conclusions: This study characterized MBP and OPN as two EOS-derived pro-angiogenic mediators found in the allergic inflammatory milieu. Our findings therefore provide a further strong link among EOS, allergic inflammation and angiogenesis.

This project was funded by a grant from the Aimwell Charitable Trust and the David R. Bloom Center of Pharmacy at The Hebrew University of Jerusalem.

INVITED SPEAKER ABSTRACTS

EOSINOPHIL ASSOCIATED TISSUE REMODELING IN PEDIATRIC EOSINOPHILIC ESOPHAGITIS

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Background: We have shown that tissue remodeling consisting of lamina propria fibrosis, angiogenesis, and vascular activation occurs in the esophagus of pediatric patients with eosinophilic esophagitis (EE). In addition, eosinophil derived pro-fibrotic factors promote tissue remodeling. However, changes in these remodeling features following topical corticosteroid therapy and their association with clinical disease features have not been systematically addressed.

Objective: To assess the features of eosinophil induced esophageal remodeling and their relationship to clinical symptoms and therapeutic response.

Methods: Features of remodeling including fibrosis, TGFβ and pSmad2/3 positive cells, as well as vascularity were assessed in the esophageal lamina propria of pediatric patients with eosinophilic esophagitis (diagnosed as epithelial eosinophils >20 per high power field) using a standardized fibrosis score and quantitative immunohistochemistry and immunofluorescence. The numbers of mast cells, which may also contribute to remodeling, were additionally assessed. The features of remodeling were correlated with clinical symptoms.

Results: Children with EE but without strictures had increased fibrosis, increased numbers of TGFβ positive and pSmad2/3 positive cells, and increased vascularity with vascular activation as compared with normal children ($p < 0.05$ for all). Following therapy, lamina propria eosinophils, fibrosis, TGFβ and pSmad2/3 expression were all decreased in those patients who had decreased epithelial eosinophils ($p<0.05$ for all). In contrast, other inflammatory cells, such as mast cells, were not decreased in the subepithelial space following therapy. The features of fibrosis and lamina propria eosinophils were positively correlated with endoscopic features of linear furrows/esophageal thickening ($r=0.64$, $p<0.05$) and symptoms of dysphagia using our standard symptoms assessment and histological scoring tools ($r=0.45$, $p<0.05$).

Conclusions: Fibrosis and eosinophil derived pro-fibrotic factors are features of eosinophilic esophagitis that can improve in those patients who have an epithelial disease resolution following therapy. Features of tissue remodeling can correlate with both endoscopic features and clinical symptoms in pediatric EE patients.

This project was funded by the Junior Women in Allergy Grant, American Academy of Allergy, Asthma, Immunology, American Partnership for Eosinophilic Disorders, HOPE Junior Faculty Research Grant

INVITED SPEAKER ABSTRACTS

EOSINOPHIL-INDUCED DAMAGE TO PARASYMPATHETIC NERVES

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Peripheral nerves recruit eosinophils via expression of chemotactic factors and adhesion molecules. As a result, eosinophils congregate around airway nerves in asthma, rhinitis and atopic dermatitis. Around nerves eosinophils release major basic protein, an endogenous antagonist for neuronal M2 muscarinic receptors that normally inhibit ACh release from parasympathetic nerves. Blockade of these M2 receptors is a mechanism for airway hyperreactivity in antigen challenged, virus infected and ozone exposed animals. Eosinophils have additional effects at nerves. Incubation of eosinophils with isolated parasympathetic nerves increases expression of substance P, while incubation of eosinophils with sensory nerves increases axon length and nerve cell branching. Thus, eosinophils increase neurotransmitter release, change neurotransmitter content and alter nerve physiology, all of which can increase and change neural activity.

An obvious question is why nerves express chemotactic factors and adhesion molecules for eosinophils if the result is enhanced neural activity and disease. Thus, it may be relevant that we have recently shown depleting eosinophil has detrimental effects. Ozone inhalation increases airway reactivity to vagal nerve stimulation that lasts 3 days in guinea pigs. One day after ozone, acetylcholine release from parasympathetic nerves is increased due to eosinophil mediated blockade of neuronal M2 muscarinic receptors and depleting eosinophils prevents hyperreactivity. In marked contrast, depleting eosinophils significantly worsened airway hyperreactivity 3 days after ozone inhalation. This corresponded to replacement of resident airway eosinophils with newly formed eosinophils by day 3 in ozone exposed animals (but not air exposed controls), as demonstrated using BrdU labeling. Furthermore, these eosinophils had a much higher rate of positive staining for nerve growth factor, and mRNA levels for CCR3, MBP1, MPP2, and EPO than any eosinophils in air exposed controls. Multiplying the number of new eosinophils times the increased expression of these cytokines suggests that new eosinophils markedly alter the local environment of nerves 3 days post ozone. Thus, phenotypically different eosinophils populate the airways 3 days after ozone exposure, and these cells have beneficial or reparative effects suggesting a physiological role for eosinophils at nerves that may be changed to a pathological interaction in disease.

This project was funded by grants from the National Institutes of Health.

INVITED SPEAKER ABSTRACTS

AIRWAY HYPERSENSITVITY INDUCED BY EOSINOPHIL GRANULE-DERIVED CATIONIC PROTEINS

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Vagal bronchopulmonary C-fiber sensory nerves play an important role in the manifestation of airway hypersensitivity associated with various airway inflammatory diseases. Activation of these nerves was suggested to be involved in the bronchial hyperresponsiveness induced by eosionophil granule-derived cationic proteins, but direct evidence was lacking. To test this hypothesis, we have recently carried out a series of studies to determine the responses of these pulmonary afferents to cationic proteins and to investigate the mechanisms possibly underlying these effects. Our results showed:

1) Intratracheal instillation of either human eosinophil granule-derived cationic proteins (e.g., major basic protein) or synthetic cationic proteins (e.g., poly-L-lysine, PLL) induced a sporadic but intense discharge of pulmonary C fibers and greatly enhanced the sensitivities of these afferents in anesthetized rats. The stimulatory and sensitizing effects of these proteins were completely nullified when their cationic charges were neutralized by mixing with polyanions (e.g., poly-L-glutamic or poly-L-aspartic acid) before delivery.

2) After the cationic charges were completely removed from PLL by succinylation, the succinylated PLL no longer produced any change in either the baseline activity or the responses to capsaicin in pulmonary C-fiber afferents.

3) In addition, eosinophil-derived cationic proteins induced a direct and long-lasting (> 60 min) sensitizing effect on the responses of isolated rat pulmonary sensory neurons to capsaicin challenge and to electrical stimulation.

4) Furthermore, these cationic proteins produced a significant inhibition of the sustained delayed-rectifier voltage-gated K^+ current and the A-type, fast-inactivating K^+ current, which accounted, at least in part, for the hyperexcitability of pulmonary chemosensitive neurons. In summary, these studies have clearly demonstrated a direct, charge-dependent, and long-lasting sensitizing effect of eosinophil-derided cationic proteins on pulmonary C-fiber sensory nerves, which may play an important part in the manifestation of airway hypersensitivity and hyperresponsiveness associated with eosinophil infiltration in the airways.

This project was funded by grants from the National Institutes of Health, USA.

INVITED SPEAKER ABSTRACTS

HELMINTH MODULATION OF ALLERGIC INFLAMMATION: NOVEL MECHANISMS AND MOLECULES

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Allergic disorders arise from a dysregulation in type-2 immunity. These type 2 responses that are aberrant in allergic conditions are induced in individuals infected with parasitic helminths. Thus allergic individuals also present with the diagnostic hallmarks of parasitic infection of high serum IgE and eosinophilia. In addition, infection with certain worm parasites stimulate regulatory responses, in particular high levels of IL-10, that are correlatively associated with suppression of allergic responses in humans. In experimental mouse models, we have shown that infection with Schistosoma mansoni can prevent anaphylaxis and allergic airway inflammation. Here the mechanisms that helminths have evolved to suppress allergic inflammation will be presented.

This project was funded by a grant from Science Foundation Ireland.

INVITED SPEAKER ABSTRACTS

THE FUNCTION OF DENDRITIC CELL SUBSETS IN ALLERGIC ASTHMA AND INFLUENZA

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The field of DC biology is currently where T cell biology was 30 years ago. It is increasingly clear that various functions like antigen uptake, processing, migration, induction of T effector responses, and induction of tolerance are not characteristics of all DCs. When studying lung DC biology, it is therefore imperative that one uses various models that are seemingly unrelated to get a full grasp of what DCs can and cannot do. We have applied this strategy using allergic asthma and influenza virus infection as two opposite disease models affecting the lungs, and have applied various transgenic strategies to deplete lung DC subsets.

Allergic asthma is characterized by airway wall infiltration with eosinophils, mast cells and Th2 cells that lead to goblet cell hyperplasia, bronchial hyperreactivity and airway wall remodelling. The ways in which Th2 cells get activated during sensitization and during recall responses have been intensively studied. Antigen-presenting dendritic cells are crucial not only in the initiation of T cell responses, but also for their maintenance. It is clear now that different DC subsets perform different tasks. Plasmacytoid DCs are able to dampen inflammation via induction of Treg cells, whereas inflammatory monocyte derived DCs promote Th2 responses. We have found that endogenous danger signals like ATP and uric acid control the activation of DCs in response to allergen challenge or in response to Th2 adjuvants, that are commonly used for inducing experimental asthma, like the Th2 adjuvant alum.

Influenza virus infection in mice leads to significant weight loss and immunopathology to the lungs. Virus is cleared by CTL responses to the virus and long lived immunity is mediated by humoral responses. Although dendritic cells (DCs) play an important role in mediating protection against influenza virus, the precise role of lung DC subsets such as CD11b- and CD11b+ conventional DCs or plasmacytoid DCs in different lung compartments is currently unknown. Early after intranasal infection, tracheal CD11b-CD11chi DCs migrated to the mediastinal LN, acquiring costimulatory molecules in the process. This emigration from the lung was followed by an accumulation of CD11b+CD11chi DCs into the trachea and lung interstitium. In the mediastinal nodes, the CD11b+ DCs contained viral nucleoprotein abundantly, but these cells failed to present Ag to CD4 or CD8 T cells, whereas resident CD11b-CD8α+ DCs presented to CD8 cells and migratory CD11b-CD8α- DCs presented to CD4 and CD8 T cells. When lung CD11chi DCs and macrophages or langerin+CD11b-CD11chi DCs were depleted using either CD11c-DTR or langerin-DTR mice, the development of virus specific CD8+ T cells was severely delayed, which correlated with increased clinical severity and a delayed viral clearance. Plasmacytoid 120G8+ CD11cint DCs also accumulated in the lung and lymph nodes carrying viral nucleoprotein but in their induced absence, there was no effect on viral clearance or clinical severity. Rather, in pDC-depleted mice, there was a reduction in antiviral antibody production following lung clearance of the virus. The precise contribution of CD11b+Ly6C+ DC subsets was in the maintenance of inducible BALT structures, sites of germinal center formation and class switching to IgA. This suggests that multiple DCs are endowed with different tasks in mediating protection against influenza virus.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS IN INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel diseases are characterized by increased numbers of leukocytes into intestinal mucosa that occur in combination with associated symptoms. A mixed inflammatory picture is often observed that includes neutrophils, lymphocytes, monocytes and eosinophils. To date, the role of eosinophils in health and in disease remains unknown and most investigations into their role stem primarily from allergic diseases and parasitic infections. This paucity of data makes it even more difficult to discern a role for eosinophils in inflammatory bowel diseases because, unlike the lung or the skin, eosinophils are resident leukocytes in the gut and increase in disease states; this supposes that an intricate system must regulate their migration and numbers. Eosinophils are equipped with the machinery to participate in gastrointestinal inflammation and in the susceptible milieu; they may initiate or perpetuate inflammation. A increasing body of literature characterizes GI eosinophilia where eosinophils can potentially interact with other resident cells leading to intestinal remodeling, mucus production, epithelial barrier, cytokine production, angiogenesis and neuropeptide release. These studies suggest eosinophils impact mucosal gastrointestinal inflammation, but definitive roles for eosinophils in inflammatory bowel diseases await discovery.

INVITED SPEAKER ABSTRACTS

RESIDENT INTESTINAL MACROPHAGE REGULATION OF EOSINOPHIL INFLAMMATION IN IBD

Amanda Beichler[1], Richard Ahrens[1], Luqman Seidu[1], Carine Blanchard[1], Rebecca Carey[2], Elizabeth Forbes[1], Maria Lampinen[3], Tara Wilson[2], Elizabeth Cohen[1], Edgar Ballard[4], Ariel Munitz[1], Huan Xu[5], Nancy Lee[6], James, J. Lee[6], Marc. E. Rothenberg[1], Lee Denson[2], Simon P. Hogan[7]

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Clinical and experimental studies have demonstrated a link between eotaxin-1, eosinophils and the inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC). We have recently performed experimental and clinical analysis to define the molecular pathways associated with eosinophil recruitment and effector in IBD. In experimental analysis we have demonstrated that dextran sodium sulphate (DSS) treatment of mice strongly upregulated colonic eosinophil levels and this correlated with disease severity. Analysis of eosinophil deficient mice defined an effector role for eosinophils in disease pathology. DSS-treatment of eotaxin-2$^{-/-}$ and eotaxin-1/2$^{-/-}$ mice demonstrated that eosinophil recruitment was dependent on eotaxin-1. In situ and immunofluoresence analysis identified eotaxin-1 expression to be restricted to intestinal F4/80^{+} CD11b^{+} macrophages (MΦ's) in DSS-induced epithelial injury. Further *in vitro* and *in vivo* analyses has identified resident intestinal M2-like MΦ as the primary source of eotaxin-1. We have recently confirmed these experimental observations in a cohort of pediatric UC patients with active disease. We demonstrate elevated levels of eosinophils in rectosigmoid colonic biopsy samples of UC patients as compared to normal controls (NL). Notably, eosinophil levels positively correlated with the UC disease histological score. Consistent with our experimental analysis, colonic eosinophilic inflammation in pediatric UC, was linked with the selective increased expression of the eosinophil chemoattractant eotaxin-1 and correlated with tissue eosinophil levels. Finally, we identified CD68^{+} intestinal MΦ's to be the primary source of eotaxin-1 in pediatric UC. These data demonstrate that intestinal macrophage derived eotaxin-1 plays a critical role in the regulation of eosinophil recruitment in colonic eosinophilic disease such as pediatric UC and provides basis for targeting the eosinophil/eotaxin-1 axis in UC.

This project was supported in part by Crohn's Colitis Foundation of American Career Development Award 2007 (S.P.H); Digestive Disease Health Center Pilot and Feasibility Research Grant of the NIH-supported Cincinnati Children's Hospital Research Foundation Digestive Health Center (1P30DK078392-01) (S.P.H), and NIH grant R01 DK058259 (L.A.D.).

INVITED SPEAKER ABSTRACTS

YOU EAT WHAT YOU WERE: EOS, DAMPS, AND CANCER

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Background: The local microenvironment within the tumor and at distant sites dictates the conditions of tumor growth, driven by the production of so called damage associated molecular pattern molecules [DAMPs] released from tumors during stress and potently recruiting eosinophils. The premetastatic niche develops from recruited hematopoietic cells [1] during tumor onset and progression, likely due to necrosis and "metastatic" inflammation [2,3]. The importance of site-specific inflammatory events as modulators of metastasis and tumor growth highlights the role of the eosinophil (Eos) [4]. Inflammation associated with chronic allergic respiratory provocation alone enhances metastasis to the lung in a murine tumor model. Asthma among patients with lung metastases is approximately two-fold higher than that observed among non-asthmatic breast cancer patients. Thus distant inflammatory events independent of or directly attributable to tumor necrosis, significantly contributes to the "seeding" of metastatic cells regardless of their intrinsic genotype. Eos are found at increased numbers within necrotic tumors [5-7].

Objectives: We wished to determine if the prototypic DAMP, HMGB1 enhances Eo survival and acts as a chemoattractant.

Methods: Eos were isolated by negative selection from human blood and used in chemotactic assays. HMGB1 from natural [liver; 293 cell line] and rHMGB1 was used. Results: DAMPs from tumor cells induce Eo degranulation (release of major basic protein and eosinophil peroxidase) and enhance their oxidative burst while oxidized DAMPs lose their stimulatory capacity. High mobility group protein B1 (HMGB1), a prototypic DAMP, released following necrosis but not apoptosis induced a similar effect on Eos. Eos express an HMGB1 receptor, the receptor for advanced glycation end product (RAGE). Of all tested biologic activities Eos respond sensitively to DAMPs with peroxide generation.

Conclusions: We postulate that Eos 'sense' necrotic cell death, migrating to and responding to areas of tissue injury/necrosis. Oxidized DAMPs lose their biologic activity when compared with native DAMPs. Eo-associated modulation of immunity within tumor and other damaged tissues may be primarily by promoting oxidative degradation of DAMPs. Novel therapeutic strategies may be considered by advancing oxidative denaturation of DAMPs by Eos or other aerobic strategies.

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This project was funded by a grant from the NIH 1 P01 CA 101944-04 (Michael T. Lotze) Integrating NK and DC into Cancer Therapy National Cancer Institute, USA.

INVITED SPEAKER ABSTRACTS

EOSINOPHIL DEFICIENCY COMPROMISES PARASITE SURVIVAL IN CHRONIC NEMATODE INFECTION.

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Background: Eosinophilia is a prominent feature of the immune response to parasitic nematodes. Larval stages can be damaged or killed by toxic products released by eosinophils *in vitro*. These observations have engendered the widely held view that eosinophils protect the host against worm infection, although the benefit of eosinophils in preventing worm infection *in vivo* has been poorly documented. The mouse is a natural host for *Trichinella spiralis*, a worm that establishes chronic infection in skeletal muscle. Recently we used mice in which the eosinophil lineage is ablated to show that eosinophils appear to protect, rather than attack muscle stage *T. spiralis*. Larval killing is mediated, in part, by an iNOS-dependent mechanism.

Objectives: Our goal is to elucidate the regulatory influence of eosinophils that prevents larval death during chronic infection by *T. spiralis.*

Methods: We have evaluated the outcome of *T. spiralis* infection in two mouse models of eosinophil ablation, ΔdblGATA$^{-/-}$ and PHIL. Quantitative RT-PCR was used to characterize the macrophage phenotypes at sites of infection, infiltrating cells were investigated by flow cytometry, and parameters of parasite development and survival were investigated.

Results: We have found that PHIL mice differ from BL/6 mice in terms of the phenotypes of local macrophages and neutrophils, as well as the numbers of infiltrating lymphocytes. Furthermore, larvae are compromised as early as day 17 of infection but are not attacked beyond day 28.

Conclusions: Our data suggest that *T. spiralis* larvae are susceptible to killing during a limited period and that the influence of eosinophils is apparent early during establishment of chronic infection. The results are consistent with a role for eosinophils in regulating immunity during the initiation of the chronic phase of *T. spiralis* infection.

This project was funded by grant NIH AI14490 to JAA and was performed in collaboration with Jamie and Nancy Lee of the Mayo Clinic.

INVITED SPEAKER ABSTRACTS

NOVELTIES IN CHURG-STRAUSS SYNDROME DIAGNOSIS AND MANAGEMENT

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Background: Churg Strauss Syndrome (CSS) is a rare vasculitis characterized by asthma, sinusitis, peripheral eosinophilia >10%, pulmonary infiltrates and eosinophilic vasculitis. Treatment options are limited and have many side effects. Interleukin(IL)-5 is a cytokine that regulates eosinophils and can be elevated in CSS. Mepolizumab (MEPO) is a humanized monoclonal anti-IL5 antibody that decreases steroid dose in subjects with non-CSS hypereosinophilic syndromes.

Objectives: This study aimed to assess whether anti-IL5 could provide CSS patients a therapy that could decrease disease activity and permit tapering of systemic steroids.

Methods: This open label pilot study enrolled 7 patients on a stable prednisone dose. Subjects received MEPO IV 750mg q4 weeks x4. A predetermined steroid tapering schedule was initiated 2 weeks into therapy. CSS activity was monitored by a number of objective measures.

Results: In all 7 patients, MEPO resulted in reduced eosinophil counts and allowed for safe reduction in steroid dosing. Upon cessation of MEPO, CSS manifestations recurred, resulting in increased steroid bursts.

Conclusions: MEPO is a safe adjunct therapy which allows for steroid tapering, while maintaining clinical stability in CSS. Anti IL5 and other targeted biologics may prove useful in the management of this challenging syndrome.

This project was funded by a grant from GlaxoSmithKline.

INVITED SPEAKER ABSTRACTS

WHAT DOES GENETICS AND PROTEOMICS TEL US ABOUT THE ROLE OF EOSINOPHILS IN DISEASE?

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The biological functions of the human eosinophil granulocytes are probably to a large extent determined by the activities of the secretory granule proteins ECP, EPX/EDN, EPO and MBP. These four major eosinophil granule proteins are truly multi functional with potent cytotoxic and non-cytotoxic activities. High concentrations of the proteins are found in blood, but also in local tissues and secretions, which imply roles in many different conditions and diseases. However, the knowledge of the actual functions and importance in vivo of these proteins in humans is unclear. Recent studies have shown several single nucleotide polymorphisms (SNPs) in the genes of these proteins, some of which alter the function of the protein and some the production. Thus, the ECP434(G>C) SNP results in the loss of the cytotoxic activity of the protein, the ECP562(G>C) and the EPX/EDN405(G>C) SNPs in the altered cellular content of the proteins. In population-based studies these SNPs were closely associated to several disease manifestations in which eosinophils classically are supposed to take part i.e. in allergy/asthma and parasite disease. These findings support our notion of the biological importance of these secretory proteins and show that one way to find out about their roles is the search for genetically modified proteins. Another approach is the study of the proteomics of the granule proteins. Indeed, ECP is present in the granules in many different forms determined largely by differences in glycosylations as determined by SELDI-Tof. As shown recently such posttranslational modifications have an impact on the cytotoxic activity with the majority of the protein (>80%) present in heavily glycosylated forms lacking cytotoxic activity. After secretion, however, the majority of secreted ECP is less glycosylated and acquires the glycosylation pattern of cytotoxic ECP. Thus, the stored ECP is inactivated by glycosylations and the secreted ECP becomes deglycosylated and activated as part of the secretion process. Two dimensional electrophoresis of the proteins of eosinophils followed by identification of 98 proteins by mass spectrometry showed other intriguing results which may shed light on the activities of the human eosinophil. One was the massive alteration of the heavy chain of EPO in eosinophils of allergic subjects, which may have its counterpart in previous demonstrations of reduced chemiluminescense production of eosinophils harvested from allergics during pollen exposure. Thus, it is our belief that the study of the genomics and proteomics of eosinophil granule proteins will help us understand the role of the human eosinophil in disease.

INVITED SPEAKER ABSTRACTS

UPDATE ON TYROSINE KINASE INHIBITORS IN CHRONIC EOSINOPHILIC LEUKEMIA

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Over the past years, our understanding of the molecular genetics of 'chronic eosinophilic leukemia' (CEL) has largely improved. The discovery of rearrangements of *PDGFRA*, including the cryptic *FIP1L1-PDGFRA* fusion, as well as the identification of abnormalities involving the *PDGFRB* and *FGFR1* genes contributed significantly to our understanding of myeloid malignancies with eosinophilia. As a consequence a novel WHO classification was introduced, and these diseases are now referred to as 'myeloid neoplasms associated with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*'.

The exquisite response of patients with *PDGFRA* or *PDGFRB* rearrangements to imatinib (Gleevec, Glivec) treatment, underscores the importance of identifying the underlying molecular lesions to ensure an optimal choice of therapy. Imatinib is still the therapy of choice for CEL patients with PDGFRA or PDGFRB rearrangements, since these oncogenic proteins are sensitive to low dose imatinib treatment (< 400 mg / day) and imatinib shows a low toxicity profile. However, despite the molecular remission observed in most imatinib treated patients, not all leukemia cells can be eradicated by kinase inhibitor treatment alone, and thus life-long low dose imatinib treatment is required.

In contrast to chronic phase CML, where a significant percentage of patients relapse during imatinib treatment due to the acquisition of resistance mutations, resistance to imatinib has not been observed in CEL (in the chronic phase). CEL can, however, progress to an acute leukemia, and in these patients response to imatinib treatment is usually of short duration due to the development of resistance. Several resistance mutations have been described, of which the T674I and D842V mutations are the most common mutations. Since these mutations are extremely rare, it is unlikely that specific programs will be initiated to develop drugs that can inhibit these mutant forms of PDGFRα, but we can take advantage of the fact that there are a large number of kinase inhibitors available that may show activity against PDGFR and these mutant forms. Using this strategy, we identified sorafenib (a BRAF kinase inhibitor) as a potent inhibitor of the T674I mutant form of FIP1L1-PDGFRα.

Today, a large number of kinase inhibitors are available with activity against PDGFRA, PDGFRB and/or FGFR1. It can be expected that over the next years treatment options for imatinib intolerant or imatinib resistant patients may further improve, and that FGFR1 inhibitors may also find their way to the clinic.

INVITED SPEAKER ABSTRACTS

CHRONIC RHINOSINUSITIS AS A CARDINAL EOSINOPHILIC DISEASE OF THE HUMAN AIRWAYS

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Among eosinophilic diseases, chronic rhinosinusitis (CRS) is striking for the high level of tissue eosinophilia. Previous studies from our laboratories and those of others have implicated chemokines and endothelial activation in the eosinophilia of CRS. Among sinonasal tissues from patients with CRS, nasal polyps tend to have the highest levels of eosinophils, suggesting that recruitment and activation of eosinophils may be important in the pathogenesis of polyp formation in this disease, a view supported by recent success with anti-IL-5 antibodies. In our recent studies, we have begun to evaluate the presence of B lymphocytes and IgA in CRS tissue, reasoning that IgA is likely to be an important activator of eosinophils in CRS, and possibly a cofactor in the role of eosinophils in pathogenesis of this disease. Levels of both tissue and secretory IgA are significantly elevated in the sinonasal tissue of patients with CRS (approximately 3-5 fold). In addition, tissue from patients with polypoid CRS have highly elevated levels of B lymphocytes as well as the TNF family member that activates B cells, BAFF (TNFSF13). In addition, tissues from polypoid CRS patients have elevated levels of two chemokines that attract B cells, namely CXCL12 and CXCL13 (SDF-1a and BLC). BAFF is likely to be involved in activation of class switch recombination of B cells to produce IgA, and the B cell active chemokines are likely to be involved in the recruitment of B cells to the upper airways. We have thus identified two pathways that are likely to be involved in the recruitment and activation of B cells that produce IgA, a primary activator of eosinophils.

This work was supported by grants from the NIH and the Ernest S. Bazley Trust.

INVITED SPEAKER ABSTRACTS

PATHOGENESIS OF EOSINOPHILIC SKIN DISORDERS

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Background: Eosinophils are associated with many cutaneous diseases and are easily recognized because of their coarse cytoplasmic granules. Investigations characterizing eosinophils and their constituents in tissue show that eosinophils and their secretory products are present and likely active in the pathogenesis of eczematous, urticarial and edematous, bullous, and vasculitic cutaneous diseases.

Objectives:
1. To delineate skin diseases in which eosinophil involvement is recognized;
2. To discuss mechanisms of eosinophil attraction to and activity in skin.

Methods: Using immunostaining for eosinophil granule proteins, intact eosinophils and extracellular granule protein deposition were indentified and their patterns compared. Using the IgE-mediated late phase reaction as a model for eosinophil infiltration in skin, current understanding of mechanisms involved in eosinophil activity is reviewed.

Results: Staining for eosinophil granule proteins reveals different patterns of eosinophil involvement including prominent deposition in the absence of intact cells. Among the cytokines that influence eosinophil involvement in skin, the eotaxins have considerable effects. Mast cells and lymphocytes are important cells in eosinophil recruitment.

Conclusions: Factors determining eosinophil infiltration and activation in tissue involve at least three interrelated signals: 1) chemoattractants; 2) adhesion molecules; and 3) cytokines and chemokines including RANTES, IL-5, GM-CSF and, importantly, eotaxins. The biological activities of eosinophils, their presence and anatomic positioning, and, notably, the effects of granule products on cells and tissues, implicate these cells in the pathogenesis of several skin diseases. An unresolved issue is how various signals affecting eosinophils in skin diseases account for the observations that eosinophils commonly disrupt and deposit toxic products through cytolysis, becoming morphologically unrecognizable with standard tissue stains.

INVITED SPEAKER ABSTRACTS

LESSONS LEARNED FROM ANTI-IL5 CLINICAL TRIALS IN ASTHMA

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The role of the eosinophils as a key player in the pathophysiology of asthma has been debated despite evidence that the cells are present and activated in the airway lumen and tissue of patients with current asthma, are increased in number when asthma is uncontrolled and decreased when asthma is controlled, and treatment strategies that aim to control airway eosinophilia are significantly more effective and less expensive in improving asthma control and decreasing asthma exacerbations compared to guideline-based clinical strategies.

Cynicism was fuelled by observations that in murine models of allergic sensitization, airway hyperresponsiveness could be induced without eosinophils. Skepticism grew stronger when therapy using monoclonal antibodies against IL-5, which has no known clinically relevant biologic activity other than on eosinophil, failed to demonstrate improvement in asthma outcomes despite decreasing airway and blood eosinophil numbers. The molecule did not reduce allergen-induced airway constriction or hyperresponsiveness, symptoms, airflow limitation or exacerbations. The most likely explanations for this apparent paradox are inappropriate methodology, inadequate sample size, or an inadequate reduction in bronchial mucosal eosinophil numbers.

The most likely explanation is the failure to include patients with significant baseline airway eosinophilia. It is intuitive that an anti-eosinophil drug will be effective only in the presence of airway eosinophils. Two recently published studies, which selected patients with severe asthma who had eosinophils in their sputum at the time of randomization, demonstrated that anti-IL5 therapy decreased asthma exacerbations, reduced the need of prednisone and improved quality of life. There was a modest improvement in airflow obstruction despite many patients having chronic airflow obstruction. More importantly, despite reducing prednisone, these patients did not have any worsening of airflow obstruction. Exhaled nitric oxide, another putative marker of airway eosinophilic inflammation, was unaffected in both clinical trials.

These studies illustrate a number of important principles. Firstly, they confirm that eosinophils are important in the pathophysiology of asthma. Secondly, since the nature of airway inflammation can change over time in the same patient, anti-eosinophil treatment will be effective only when eosinophils are present in the airway. Thirdly, luminal eosinophils represent biologically active cells. Fourthly, clinical trials with small numbers of carefully characterized patients can demonstrate biologically relevant mechanisms, where large studies have not. Finally, monitoring of exhaled nitric oxide is not effective in directing anti-eosinophil therapy in patients with severe asthma and airway eosinophilia.

Supported by Canada Research Chair Program and GlaxoSmithKline

TREATMENT WITH A MONOCLONAL ANTIBODY AGAINST IL-5 (MEPOLIZUMAB) REDUCES SEVERE EXACERBATIONS IN PEOPLE WITH REFRACTORY ASTHMA

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Asthma is a disease characterised by variable airflow obstruction (VAO), airway hyperresponsiveness (AHR) and airway inflammation which is usually but not always eosinophilic. However within this definition there is a considerable heterogeneity in the clinical presentation, physiology and pathology of the disease. This relates to heterogeneity in response to treatment. In order to take full advantage of the new biological therapeutic agents that are becoming available to treat asthma and related diseases we need to understand this phenotypic complexity and relate it to pathogenesis and treatment response.

Two of the principle components of asthma are airway dysfunction and eosinophilic inflammation. Airway dysfunction is responsible for most of the symptoms of asthma including episodic breathlessness, wheeze and chest tightness. In contrast eosinophilic airway inflammation is often clinically silent. Measures of airway dysfunction such as FEV1, variability in peak flow and AHR are commonly used as the outcome measures in clinical trials of new asthma medications. It has been thought that eosinophilic airway inflammation and airway dysfunction were closely related. However while they commonly co-exist in the same patient there is a weak to non-existent correlation between the degree of airway dysfunction and the severity of airway inflammation, at least as measured by the sputum differential count. In addition inflammation and dysfunction can often be dissociated as for example in eosinophil bronchitis a common cause of cough with a raised sputum eosinophilia but no airway dysfunction, and non-eosinophilic asthma with airway dysfunction but no eosinophils.

The role of eosinophils in asthma has been debated for several decades with concepts ranging from amelioration to a primary effector cell in causing tissue damage in the disease. The awareness that eosinophils were neither necessary nor sufficient to develop asthma and disappointing results from trials with anti-IL-5 mAb's which reduced eosinophils without effecting airway dysfunction meant that doubt was cast on their tissue damaging role. However a number of studies have shown that an airway eosinophilia tracks most closely with the important phenotype of severe exacerbations which can lead to hospital admissions and death. An association doesn't mean causation.

We therefore undertook an investigator led, 12 month, single centre, double blind placebo controlled trial of an anti-IL-5 mAb mepolizumab, in patients with eosinophilic asthma and a history of severe exacerbations. The primary outcome was severe exacerbations. We demonstrated a significant reduction in the number of severe exacerbations in the treatment group with about a 40% difference between placebo and active treatment. However there was no effect on measures of airway dysfunction or the asthma control questionnaire emphasising the dissociation between the airway dysfunction and inflammatory phenotypes. The extent to which there was a reduction in exacerbations tracked the degree to which mepolizumab reduced the airway eosinophilia suggesting that a drug which was more effective at reducing the tissue eosinophilia (only reduced by about 50% by mepolizumab), would be even more effective at reducing exacerbations.

This study is the strongest evidence so far that eosinophils play a tissue damaging role in asthma and suggest that anti-eosinophil strategies have an important role in treating severe exacerbations in asthma.

INVITED SPEAKER ABSTRACTS

TARGETED IMMUNE-BASED THERAPIES FOR THE TREATMENT OF EOSINOPHILIC DISORDERS

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Peripheral blood and/or tissue eosinophilia is associated with a wide range of clinical disorders, including asthma, hypersensitivity reactions, helminth infections, neoplasia, and hypereosinophilic syndrome. In many of these, the role of the eosinophil in disease pathogenesis remains controversial. The development of monoclonal antibodies and other immunologically-based therapies targeting molecules involved in eosinophilia and eosinophil activation has not only led to novel therapeutic interventions for eosinophil-associated disorders, but has provided a means to dissect the role of eosinophils in the clinical manifestations of these conditions. Potential targets and available agents will be reviewed with examples given from recent clinical trials.

INVITED SPEAKER ABSTRACTS

NOVEL THERAPEUTIC TARGETS IN EGIDs

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Eosinophilic gastrointestinal disorders (EGIDs) can successfully be controlled with diet modification or steroids. However, a significant portion of the patient population is not responsive to these therapies emphasizing the importance of uncovering potential new targets. Indeed, some recent studies have emphasized the beneficial use of targeted antibody therapy such as anti-IL-5 in EGIDs. The understanding of the molecular events involved in the inflammatory processes occurring in EGIDs (from sensitization to cell infiltration and tissue remodeling), is necessary for the development of new therapeutics and possible targets will range from cell development and maturation, to recruitment, activation and adhesion.

This project was funded by the PHS Grant P30 DK0789392 (C.B.), the NIH AI079874-01 (C.B.), AI070235, AI45898, and DK076893 (M.E.R.), the Food Allergy and Anaphylaxis Network (M.E.R.), Campaign Urging Research for Eosinophil Disorders (CURED), the American Partnership for Eosinophilic Disorders (AFPED Junior faculty HOPE research grant), the Buckeye Foundation (M.E.R.) and The Food Allergy Project (M.E.R).

INVITED SPEAKER ABSTRACTS

ANTI-IL-5R ANTIBODIES FOR EOSINOPHIL-ASSOCIATED DISEASES

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Background: Eosinophils are important contributors to many inflammatory processes in asthma and hypereosinophilic syndromes. A number of factors can affect eosinophil production, survival, recruitment, and activation, including IL-5, which is central to many of these processes and a target to control levels of eosinophils. Regulation of the effects of IL-5 can be achieved by a number of strategies including antibodies directed against IL-5 itself or, possibly, the receptor for IL-5, i.e. IL-5R.

Objectives: A humanized, recombinant, fucosylated, monoclonal antibody, MEDI-563, has been developed that binds to the α-chain of the IL-5R and can induce apoptosis of eosinophils via antibody-dependent cell-mediated cytotoxicity (ADCC). The objective of this presentation will be to report on the safety, pharmacology, and pharmacokinetics of MEDI-563 in human studies

Methods: MEDI-563 was given as a single dose-escalating, intravenous preparation to 44 atopic asthmatic subjects. In this phase I, open-labeled study, the safety and effects of this product were evaluated.

Results: Mean peripheral eosinophil levels were reduced in a dose-dependent fashion and remained below baseline levels throughout the study. Similarly, serum levels of ECP were reduced. Adverse events were infrequent but included a reduction in white blood cell counts in approximately one-third of the subjects.

Conclusions: The monoclonal antibodies directed against the IL-5R reduced peripheral blood eosinophil events and its use was found safe. These preliminary studies indicate another IL-5-dependent process to regulate peripheral blood eosinophils and, presumably, control the contribution of eosinophils to allergic inflammation.

This project was funded by a grant from MedImmune, Inc.

INVITED SPEAKER ABSTRACTS

SIGLECS AND OTHER INHIBITORY RECEPTORS ON EOSINOPHILS AS POTENTIAL THERAPEUTIC TARGETS

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Siglecs (sialic acid-binding, immunoglobulin-like lectins) are a family of single-pass transmembrane cell surface proteins found predominantly on leukocytes. They possess N-terminal lectin domains recognizing sialic acids. Most Siglecs have immunoreceptor tyrosine-based inhibitory motifs in their cytoplasmic domains, suggesting that these molecules function in an inhibitory capacity. The same can be said of other inhibitory receptors expressed by eosinophils, such as IRp60/CD300a, FcgRIIB, LIR3, and p140. Among the Siglecs expressed by eosinophils, we know the most about Siglec-8. On eosinophils, Siglec-8 engagement with specific antibodies results in apoptosis mediated via caspases and/or mitochondrial reactive oxygen species. The closest functional paralog in the mouse is Siglec-F, also expressed by eosinophils. Despite only modest homology, both Siglec-8 and Siglec-F preferentially recognize a sulfated glycan ligand, namely 6'-sulfo-sLe^x or NeuAcα2-3Galβ1-4(Fucα1-3)(6-O-sulfo)GlcNAc. A soluble synthetic polyacrylamide polymer displaying 6'-sulfo-sLe^x binds purified human eosinophils, and in whole blood, eosinophils are the only leukocyte subtype to detectably bind polymeric 6'-sulfo-sLe^x. IL-5-cultured eosinophils undergo apoptosis when incubated with either Siglec-8 monoclonal antibody or polymeric 6'-sulfo-sLe^x, although the glycan polymer is less effective. Experiments in normal, Siglec-F-deficient and hypereosinophilic mice have resulted in similar conclusions that Siglec-F, like Siglec-8, plays an important role in regulating eosinophil accumulation and survival in vivo. Regarding its natural ligand, ongoing studies suggest that α2, 3-linked sialic acid-containing glycoprotein Siglec-F ligands, and enzymes required for their synthesis, are constitutively expressed in mouse lung, especially by airways epithelium. Ligands for Siglec-F are increased during Th2-like forms of lung inflammation and the sialyltransferase *St3gal3* is required for constitutive expression of Siglec-F ligand. Given the resurgent interest in eosinophil-directed therapies for a variety of disorders, therapies focusing on Siglec-8 and related eosinophil inhibitory receptors could prove to be a useful adjunct to our current armamentarium for the treatment of asthma, allergies and related disorders.

This project was funded by grants AI41472 and AI72265 from the National Institutes of Health, the Human Immunology grant program of the Dana Foundation, and through a Cosner Scholar in Translational Research grant from Johns Hopkins University.

T-CELL DERIVED IFN-γ INHIBITS EOSINOPHIL DIFFERENTIATION

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Background: Hematopoietic differentiation in the bone marrow is tightly regulated by local growth factors, but can also be modulated by external factors. Especially during phases of immune activation, the bone marrow output has to be modulated and we postulate that activated leukocytes that infiltrate the bone marrow can provide important cues to regulate the haematopoietic process.

Objectives: In this study we investigated how T cell activation affects myelopoiesis in general and eosinophil formation in particular.

Methods: For this approach, we make use of a transgenic mouse model, in which the TNF-superfamily member CD70 is overexpressed on B cells, which induces strong T cell activation due to enhanced triggering through the costimulatory receptor CD27.

Results: We found that CD70-transgenic (CD70TG) mice, which have high numbers of IFN-γ producing T cells, are fully devoid of eosinophils in bone marrow, blood and spleen, whereas numbers of neutrophils and monocytes are increased. Induction of allergic airway inflammation, which typically increases eosinophil formation in the bone marrow and provokes a strong eosinophilia in the lungs of wildtype (WT) mice, fails to generate eosinophils in CD70TG mice. Analysis of the bone marrow of CD70TG mice revealed that these mice lack eosinophil-specific progenitors and *in vitro* experiments show that granulocyte/macrophage progenitors (GMPs) of these mice are unable to generate eosinophils upon culture with IL-5. We found that IFN-γ is the cause of this defect, as addition of this cytokine strongly inhibits eosinophil differentiation of WT-derived GMPs and common myeloid progenitors (CMPs) *in vitro*, while eosinophil formation is fully restored when CD70TG mice are backcrossed on an IFN-γ-/- background. Moreover, adoptive transfer studies indicate that CD27-mediated IFN-γ production by T cells is sufficient to inhibit eosinophil formation *in vivo*. Finally, detailed analysis of transcription factors important for eosinophil differentiation revealed that IFN-γ regulates the expression of PU.1 at the level of the CMPs and GMPs. PU.1 is an important transcription factor for myeloid differentiation, but counteracts the activity of GATA-1, which is essential for eosinophil differentiation, as it controls transcription of the IL-5Rα gene.

Conclusions: These data indicate that T cell derived IFN-γ can inhibit the differentiation of GMPs to eosinophils by interfering with the activity of key transcription factors.

This project was funded by a grant from NWO.

LENTIVIRAL shRNA KNOCKDOWN OF CHARCOT-LEYDEN CRYSTAL PROTEIN/GALECTIN-10 EXPRESSION IN DEVELOPING HUMAN EOSINOPHILS IMPAIRS GRANULOGENESIS

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Background: Charcot-Leyden Crystal (CLC) protein, also known as Galectin-10 (Gal-10), is expressed at high levels by both eosinophils and basophils. It is also expressed by T regulatory cells (Tregs) and is required for Treg function, since siRNA knockdown of CLC/Gal-10 results in loss of Treg suppressor activity. In the developing eosinophil, the CLC/Gal-10 gene is one of the most highly expressed at the mRNA level, and mature eosinophils continue to transcribe CLC/Gal-10 mRNA, while all of the secondary granule genes are all silenced. The function of CLC/Gal-10 in eosinophils remains indeterminate, but we have shown that it interacts with the eosinophil ribonucleases EDN and ECP in vitro, co-localizes with EDN and CD63 in IFNg-activated eosinophils, and may be involved in their secretion via vesicular transport during piecemeal degranulation.

Objectives: Our objective for the current studies was to determine if CLC/Gal-10 participates in granulogenesis, i.e. the transport and packaging of these ribonucleases into secondary (crystalloid-containing) granules, during eosinophil differentiation.

Methods: A lentiviral shRNA vector was identified that completely knocks down CLC/Gal-10 expression in AML14.3D10 eosinophils and in cord blood CD34+ progenitors induced to differentiate into eosinophils, compared to non-target shRNA controls. Knockdown was confirmed by Westerns, immunofluorescence, and the effect on eosinophil development, cell and granule morphology was determined by Fast Green/Neutral Red staining, differential cell/granule counts, and immunofluorescence confocal microscopy.

Results: Lentiviral shRNA knockdown of CLC/Gal-10 did not inhibit eosinophil progenitor cell proliferation induced by IL-5, but impaired granulogenesis, with decreased formation of Fast Green positive secondary (crystalloid) granules. The numbers of eosinophils containing secondary granules after 14 days was decreased by more than 50% compared to controls, and knockdown cells that still contained some granules, contained significantly fewer Fast Green positive granules/cell. Knockdown cells also contained increased numbers of large empty granule containers and very large empty granules, suggesting fusion of empty granule containers in the CLC/Gal-10 deficient eosinophils.

Conclusions: Lentiviral shRNA knockdown of CLC/Gal-10 in eosinophil progenitors has revealed a novel role for this highly abundant yet enigmatic member of the galectin superfamily in granulogenesis during eosinophil development. In ongoing studies, we are examining whether CLC/Gal-10 knockdown also impairs eosinophil piecemeal degranulation and secretion, via vesicular transport, of the granule cationic ribonucleases (EDN/ECP), or other proteins (MBP1/2 or EPX) in the differentiated, mature eosinophil.

This project funded by NIH grant AI25230 to SJA. CBD was supported in part by training grant T32DK007739. BTM is supported in part by training grant T32HL082547.

THE EFFECT OF OBESITY ON EOSINOPHILIC INFILTRATION IN A MURINE MODEL OF ALLERGIC ASTHMA

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Background: Increases in eosinophil (EO) numbers in the tissues, blood, and bone marrow (BM) are a hallmark of asthma and, in general, elevated numbers correlate with disease severity. Epidemiological data indicate that obesity increases the prevalence and incidence of allergic asthma (Camargo et al., 1999). Increased fat mass, particularly with central obesity, leads to production of cytokines and chemokines, such as IL-6, TNF-α and eotaxin (Vasudevan et al, 2006). Studies have shown that genetically obese mice exhibit innate airway hyperresponsiveness (Shore et al., 2003; Shore & Johnston, 2006), but little attention has been given to the allergic pulmonary EO recruitment in obese animals.

Objectives: To investigate the time-course EO influx into lungs and the role of Th2 cytokines in diet-induced obese mice.
Methods: Four-week-old male C57bl6/J mice received a high fat diet for 10 weeks. On the eighth week, mice were sensitized with two s.c. ovalbumin (OVA) injections at 7 day intervals. One week thereafter, sensitized and non-sensitized animals were intranasally challenged with OVA. The mice were killed at 48 and 72h, and EO counts in blood, bronchoalveolar lavage fluid (BAL) and BM were evaluated.

Results: High-fat diet mice exhibited a significant increase in body weight and epididymal fat, as well as increased total serum cholesterol levels compared with non-obese groups. Intranasal challenge with OVA in sensitized mice largely increased the EO counts in BAL at 48 h and 72 h post-OVA challenge (0.67±0.06 and 0.12±0.03x106/BAL, respectively). Eosinophils were nearly absent in the non-sensitized mice. The sensitized obese mice showed a delayed EO emigration to BAL, peaking at 72 h post-OVA challenge (0.2±0.04x106/BAL). In addition, the morphological analysis showed that lung parenchyma of sensitized obese mice presented a markedly higher EO infiltration at both 48 h and 72 h post-OVA challenge when compared with non-obese mice. In BM, a significant increase in counts of both mature and immature EO was also found in sensitized obese (1.73±0.32x106/ml and 0.49±0.07 x106/ml, respectively) compared with sensitized non-obese mice (0.28±0.07x106/ml and 0.15±0.05x106/ml, respectively). The levels of TNF-α, IL-6 and IL-10 significantly increased in BAL of sensitized obese mice, peaking at 72 h-post OVA challenge.

Conclusions: We have established an experimental model in C57bl6/J obese mice that clearly show a potentiation of EO influx in response to OVA challenge. In obese mice, EO are likely to be retained in the lung parenchyma exerting their effector functions in promoting the pathogenesis of airways diseases.

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This project was funded by a grant from CAPES and FAPESP.

AGONIST ACTIVATION OF EOSINOPHIL SHAPE CHANGE AND MEDIATOR RELEASE IS DEPENDENT ON RAC2 GTPASE

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Background: Tissue recruitment of eosinophils and their inflammatory mediator release contributes to allergic symptoms by causing airway hyperresponsiveness, edema and tissue inflammation. Shape changes associated with cell motility, along with release of eosinophil mediators, may be regulated by the mammalian Rho-related guanosine triphosphatases (GTPase), Rac1, Rac2, or Rac3. Of these, Rac2 plays an essential role in F-actin formation as a central process underlying cell motility, exocytosis, and respiratory burst in neutrophils. However, it is not known whether Rac1 or Rac2 is required for eosinophil shape change and activation. Our earlier studies (Fulkerson *et al.* (2005) *Blood* 106:436) indicated that Rac2 is required for eotaxin-2-induced chemotaxis in eosinophils. Here we determined whether Rac2 is a central regulator of mediator release and shape change leading to migration as part of the immune function in eosinophils.

Objectives: To determine the specific role of Rac2 GTPase, an intracellular receptor-mediated activator of F-actin formation, in eosinophil movement and mediator release.
Methods: We isolated splenic eosinophils from CD2-IL-5 transgenic mice (WT) and Rac2-deficient mice bred against the CD2-IL-5 transgenic background (Rac2 KO), and compared their ability to release superoxide (using cytochrome *c* reduction) or eosinophil peroxidase (EPO, using tetramethylbenzidine as the substrate) in response to phorbol myristate acetate (PMA) and calcium ionophore (A23187). We also examined the morphology of cells (shape change) and their actin polymerization state in response to eotaxin-2 or platelet-activating factor (PAF) by using rhodamine-phalloidin staining combined with flow cytometry and confocal microscopy analysis.

Results: Whole spleen and MACS-purified splenic eosinophils from Rac2 KO mice showed significantly decreased levels of superoxide release (37% and 26% reduced from maximal WT responses, respectively; $p < 0.05$). Rac2 KO eosinophils also showed defects in degranulation (61% reduced from WT responses to A23187; $p < 0.05$) and shape changes in response to eotaxin-2 or PAF.

Conclusions: These findings suggest that gene deletion of Rac2 reduces eosinophil motility and mediator release. However, these effects were partial, suggesting compensatory signaling through Rac1 may be occurring to promote mediator release from eosinophils. Our observations suggest that Rho GTPases are likely to be important molecular regulators of eosinophil-related diseases including allergy and asthma.

This project was funded by grants from NIH (USA), the Canadian Institutes of Health Research, The Lung Association of Alberta and NWT, and Canada Foundation for Innovation (Canada).

THE N-TERMINUS OF THE EOSINOPHIL CATIONIC PROTEIN (ECP) RETAINS MOST OF ITS BACTERICIDAL AND MEMBRANE DISRUPTION CAPACITIES

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Background: Eosinophil cationic protein (ECP) is an eosinophil secretion protein with a wide spectra antipathogen activity, suggesting a relative non-specific mechanism of action. Although there is no direct evidence of the involvement of ECP in the host response to bacterial infection, ECP kills both Gram- negative and Gram- positive cells, and its mechanisms relies on its action on both the plasma membrane and the bacterial wall.

Objectives: In a search for the structural determinants of ECP antimicrobial activity, we have identified an N-terminal domain (residues 1-45) that retains most of ECP's membrane-destabilizing and antimicrobial activities. Two sections of this domain, ECP (1-19) and ECP (24-45), have also been evaluated.

Methods: Recombinant ECP was expressed and purified using a prokaryote system. Minimal inhibitory concentration was calculated to compare the peptide bactericidal activity. Membrane depolarization activity and membrane leakage activity were followed by a fluorescence assay. Lipopolysaccharide binding was registered by the displacement of a cadaverine probe. Damage on bacteria cells was assessed by scanning and transmission electron microscopy. The peptide structural conformational changes were followed by circular dichroism.

Results: All three peptides bind and partially insert into lipid bilayers, inducing aggregation of lipid vesicles and leakage of their aqueous content. In such an environment, the peptides undergo conformational change, significantly increasing their α-helix content. The bactericidal activity of the three peptides against *E. coli* and *S. aureus* has been assessed at both the cytoplasmic membrane and the bacterial envelope levels. ECP(1-45) and ECP(24-45) partially retain the native protein ability to bind lipopolysaccharides and electron microscopy reveals cell damage by both peptides. Interestingly, the *E. coli* cells agglutination activity of ECP is only retained by the longest segment ECP(1-45).

Conclusions: The ECP(1-45) peptide reproduces most of ECP's antimicrobial properties and retains the protein capacity to agglutinate *E. coli* cells. Comparative results suggest a task distribution, whereby residues 1-19 would contribute to membrane association and disruption, while the 24-45 region would be essential for LPS binding and bactericidal action.

This project was funded by a grant from Ministerio de Educación y Cultura (Grant BFU2006-15543-C02-01/BMC) and by the "Fundació La Marató de TV3" (TV3-031110).

EOSINOPHILS MEDIATE ANTIVIRAL EFFECTS *IN VIVO* AND DIRECTLY KILL PARAINFLUENZA VIRUS *IN VITRO*

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Background: Respiratory viruses cause 80% of asthma attacks in children and 50% of attacks in adults. We previously showed that airway eosinophils are activated by viral infection, that this activation causes virus-induced airway hyperreactivity, and that eosinophils participate in virus clearance. In this project, we studied the interactions of Parainfluenza Type 1 (PIV-1) with eosinophils both *in vivo* and *in vitro*.

Objectives: In these studies, we investigated whether eosinophils mediate PIV-1 clearance *in vivo*. Based on these results, we next determined whether eosinophils are activated by PIV-1, support virus replication, and directly inactivate PIV-1 *in vitro*.

Methods: We examined virus clearance in three different animal models: in ovalbumin (OVA) sensitized-challenged mice, in mice that have interleukin-5 (IL-5) over-expressed in the lungs and increased eosinophils, and in mice which have IL-5 over-expressed in the tissues but no eosinophils due to targeted ablation of eosinophil precursors with diptheria toxin. Mice were infected for four days, and virus content in the lungs was quantified by qPCR. For the *in vitro* studies, human eosinophils were treated with or without interferon (IFN)-gamma overnight, which is known to activate eosinophils and up-regulate toll-like receptors, and infected with PIV-1. Eosinophil activation was measured by changes in cell surface CD69 expression. Virus replication in eosinophils and eosinophil-mediated virus killing were quantified by qPCR and titration in Rhesus monkey kidney cells (RMKs).

Results: Sensitized-challenged mice had a 10-fold decrease in virus load in the lungs compared to unsensitized, wild-type (WT) mice. Mice with increased levels of eosinophils in the lungs had a 10-fold decrease in virus content compared to WT littermate controls. Mice over-expressing IL-5 in the tissues had a 2-fold decrease in PIV-1 content in the lungs compared to WT, and PIV-1 levels were restored to WT levels in IL-5 over-expressing mice lacking eosinophils. Human eosinophils mediate virus killing of PIV-1 *in vitro* and pre-treatment with IFN-gamma enhances virus killing. This killing of viruses was observed by RMK assay, which measures infectious virus, but not when viral genome was measured by qPCR, suggesting that the viral RNA is not the only target of the eosinophil. IFN-gamma-treated human eosinophils up-regulate CD69 in response to virus infection. Human eosinophils are abortively infected with PIV-1.

Conclusions: Collectively, these data suggest that eosinophils participate in virus clearance *in vivo.* Human eosinophils are activated by and render PIV-1 replication-incompetent *in vitro*. In addition, human eosinophils serve as a dead-end host for PIV-1 infection.

This project was funded by a grant from the National Institutes of Health.

THE INNATE IMMUNE SHIELD: PROBIOTIC *LACTOBACILLUS* SPECIES PROMOTE EXPRESSION OF EOSINOPHIL-ACTIVE CYTOKINES AND RESISTANCE TO THE LETHAL SEQUELAE OF RESPIRATORY VIRUS INFECTION

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Background: Infection with respiratory viral pathogens, including human respiratory syncytial virus (hRSV), can induce inflammatory lung injury and result in significant morbidity and mortality. In an effort to identify and characterize inflammatory responses contributing to pneumovirus pathogenesis *in vivo*, we utilize a natural pathogen model, in which mice infected with pneumonia virus (PVM) develop pneumonia, progressing to pulmonary edema and respiratory failure, symptoms consistent with acute respiratory distress syndrome (ARDS).

Objectives: As part of our broad interests in inflammation and immunomodulatory therapy, we have recently become interested in probiotics, defined as living microorganisms or microbial products with immunomodulatory properties beneficial to the health of the host organism. Our focus is on *Lactobacilli*, probiotic microorganisms which have been shown to prevent gastrointestinal infections and to alter responses to food and systemic allergens. This study probes the effect of mucosal administration of live *Lactobacillus* strains on the PVM-host interaction and ensuing disease pathogenesis.

Methods: BALB/c mice were inoculated intranasally with live *Lactobacillus* on days 0 and 7, followed by intranasal inoculation with PVM virions on day 14. Lung tissue and bronchoalveolar lavage (BAL) fluid were collected at selected time points between days 7 and 21. Transcripts isolated from lungs were subject to PCR array analysis. Virus copy number was assessed by RT-qPCR. Eosinophils and neutrophils recruited to the airways were detected by modified Giemsa staining of BAL fluid.

Results: Inoculation of mice with either of two live, non-pathogenic lactobacillus strains (*L. plantarum*, *L. reuteri*) prior to challenge with PVM protects mice from mortality associated with the viral infection. Additionally, we demonstrated that *Lactobacillus*-treated animals recruit both eosinophils and neutrophils to the airways at an earlier time point than control-treated mice, and that granulocyte recruitment is associated with diminished virus titer in lung tissue. PCR array analysis of transcripts expressed in lung tissue at 2, 4, and 6 days after the second inoculation with *Lactobacillus* strains reveals augmented expression of a distinct set of cytokines, most notably interleukin-18, a pleiotropic cytokine produced by monocytes and macrophages that has broad impact on eosinophil recruitment, eotaxin and interferon-gamma production, and eosinophil-mediated inflammatory responses.

Conclusions: Direct contact of live *Lactobacillus* strains (specifically, *L. plantarum and L. reuteri*) with the respiratory epithelium appears to confer an effective innate immune shield, which may occur via alterations of the endogenous inflammatory responses. As significant resources and attention are devoted to combating severe respiratory infections, the ability to evoke an effective innate immune shield by mucosal contact with an otherwise benign microbial organism or component provides a promising application from the perspectives of epidemiology and biodefense.

SITE-SPECIFIC RECONSTITUTION OF EOSINOPHILS IN EOSINOPHIL-DEFICIENT (*PHIL*) MICE DIFFERENTIALLY AFFECTS T CELL ACTIVATION AND RECRUITMENT IN ALLERGIC PULMONARY INFLAMMATION

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Rationale: Allergic pulmonary inflammation depends on the activation and recruitment of T cells to the lung. The causative relationship between eosinophils and T cells remains unclear despite innumerable studies characterizing effector functions of eosinophils. Our data suggests distinct roles for pulmonary and circulating eosinophils in mediating immune modulation in the development of allergen-induced pulmonary pathologies.

Objective: To demonstrate that pulmonary and circulating eosinophils have distinct roles in activating and recruiting 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.*)) into *PHIL* or *IL-5*$^{-/-}$ mice at the time of allergen challenge using an established acute ovalbumin (OVA) sensitization/challenge protocol. T cells and eosinophils were assessed in allergen-challenged animals by flow cytometry.

Results: OVA sensitization/challenge of *IL-5*$^{-/-}$ mice failed to elicit pulmonary pathologies despite the expansion of lung draining lymph node (DLN) T cell numbers. Direct adoptive transfer (*i.t*) of eosinophils into these mice restored Th2 pulmonary pathologies, including T cell accumulation in the lung. In contrast, adoptive transfer (*i.t.*) of eosinophils into the lungs of *PHIL* mice failed to elicit the accumulation of T cells in any pulmonary compartments (i.e., airway lumen, lung tissue, or DLN). Significantly, adoptive transfer of eosinophils via peripheral circulation (*i.p.*) was able to restore DLN T cell numbers in OVA-treated *PHIL* mice to that of wild type mice but did not restore T cell accumulation in the lung. In addition, only eosinophils adoptively transferred via *i.p.* were able to migrate to the DLN and this migration is completed by a CCR7-independent mechanism.

Conclusion: Our data suggests that circulating eosinophils that reach the lymphatics are sufficient to induce proliferation of $CD4^+$ T cells in the DLN and pulmonary eosinophils mediate recruitment of T cells to the lung at the time of allergen challenge. Collectively, these studies highlight a previously under-appreciated role for eosinophils in the localized pulmonary immune responses that occur upon allergen provocation.

EOSINOPHILS INHIBIT TGF-β-INDUCED FOXP3$^+$ REGULATORY CD4$^+$ T CELLS BY RELEASING IL-4

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Background: Naive CD4$^+$ T cells differentiate into functionally distinct T helper (Th) cell subsets or into Foxp3$^+$CD25$^+$ regulatory T (Treg) cells in response to the cytokine milieu in which they encounter antigen. Treg cells play a critical role in maintaining immunological self-tolerance and regulating other classes of immune response including allergy and allograft rejection. Evolving findings have revealed that eosinophils have immunoregulatory roles as professional antigen-presenting cells and as modulators of CD4$^+$ T cell and B cell functions. Notably, eosinophils contain preformed stores of diverse cytokines including TGF-β and IL-4 that have been reported to modulate Foxp3$^+$ Treg cells. However, it has not yet been elucidated whether eosinophils play a role in regulating Foxp3$^+$ Treg cells.

Objectives: To investigate effect of eosinophils on TGF-β-induced Foxp3$^+$ Treg cell conversions from naïve CD4$^+$ T cells.

Methods: We used naive Foxp3$^-$CD4$^+$ T cells sorted by flow cytometry from spleens of mice with a bicistronic enhanced GFP reporter cloned into the endogenous *Foxp3* locus. Naïve Foxp3-GFP$^-$ CD4$^+$ T cells were activated with plate-bound anti-CD3 (2μg/ml) and soluble anti-CD28 (1μg/ml) in the presence of TGF-β (1ng/ml). To test the effect of eosinophils on the induction of Foxp3 by TGF-β, eosinophils were purified from spleens of IL-5 transgenic mice and co-cultured with Foxp3-GFP$^-$CD4$^+$ T cells for 3 days in the presence of TGF-β. In some experiments, anti-mouse IL-4 neutralizing antibody (0.5-10μg/ml) was added into the co-cultures.

Results: Three days after activation with anti-CD3/CD28, few of these Foxp3-GFP$^-$CD4$^+$ T cells expressed Foxp3-GFP. As expected, the addition of TGF-β induced Foxp3-GFP expression in a significant percentage of cells. However, this TGF-β induced Foxp3 conversion was significantly inhibited with the addition of eosinophils in an eos:T cell ratio-dependent manner. To understand the mechanism by which eosinophils mediates the inhibition of Foxp3 expression, we added anti-mouse IL-4 neutralizing antibody at different concentrations into the co-cultures, and found that the inhibition of Foxp3 expression by eosinophils was almost extinguished with increasing amount of anti-IL-4 antibody.

Conclusions: This study shows a novel finding that eosinophils may be involved in regulating Foxp3$^+$ Treg cells and further broadens the immunoregulatory roles of eosinophils as innate immune cells.

This project was funded by NIH grants AI020241 and AI051645 to P.F. Weller

HUMAN EOSINOPHILS EXPRESS MUSCARINIC RECEPTORS, PRODUCE CORTICOTROPIN RELEASING HORMONE IN THE COLON AND MAY PARTICIPATE IN THE REGULATION OF MACROMOLECULAR PERMEABILITY VIA MAST CELLS

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Background: Chronic psychological stress causes intestinal barrier dysfunction mediated by corticotrophin-releasing hormone (CRH) and mast cells. Increased intestinal permeability has been shown in patients with ulcerative colitis (UC), and active eosinophils are present in both active and quiescent UC.

Objectives: Our aim was to examine the effects of CRH on mucosal barrier function in the human colon and to elucidate the mechanisms involved in CRH-induced hyper-permeability in ulcerative colitis, focusing on the role of eosinophils.

Methods: Biopsies from 15 UC patients in remission and 15 healthy volunteers were assessed for macromolecular permeability using an Ussing chamber during modulation of CRH receptors, muscarinic receptors and mast cell stabilizer (lodoxamide tromethamine). The biopsies were examined by immunohistochemistry and by light microscopy for CRH producing cells, eosinophils and mast cells in relation to cholinergic receptor localization. Purified peripheral blood eosinophils were stimulated with carbachol and atropine, and the expression of muscarinic receptors M2 and M3 was assessed by flow cytometry. Eosinophil CRH release was measured by peptide enzyme immunoassay.

Results: Permeability to Horse Radish Peroxidase (HRP) was increased in UC patients compared to controls. The elevated HRP uptake in UC was normalized by pretreatment with atropine, the CRH receptor antagonist α-helical CRH (9–41) or mast cell stabilizer. Immunohistochemistry showed increased numbers of lamina propria eosinophils expressing CRH and the muscarinic receptor subtypes M2 and M3 in the UC group. Flow cytometry revealed expression of M2 and M3 receptors on peripheral blood eosinophils. The expression of M2 receptors decreased after incubation of the eosinophils with atropine. CRH concentrations of 8,0 $\pm$ 0,2 μmol/ml in eosinophilic supernatant were detected by EIA measurements.

Conclusions: Increased transmucosal uptake of protein antigens in the non-inflamed colon of UC involves cholinergic signalling, CRH and activation of mast cells. Subepithelial CRH-producing eosinophils expressing muscarinic receptors may be involved in regulating this process.

This project was funded by grants from the Swedish Research Council – Medicine and the Ihre Foundation.

CYSTEINYL LEUKOTRIENES ELICIT SECRETION BY HUMAN EOSINOPHIL GRANULES

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Background: Cysteinyl leukotrienes (cys-LTs) and their receptors (CysLTR) have clear roles in pathophysiological conditions such as asthma and other allergic diseases. Eosinophils are known to contain an abundance of preformed proteins stored within their cytoplasmic granules and to express both $CysLT_1R$ and $CysLT_2R$. We previously demonstrated that eosinophil granules express cytokine receptors on their membranes and can function, upon extrusion from eosinophils, as independent secretory organelles releasing granule constituents in response to activating chemokines and cytokines (PNAS, 105:18478, 2008).

Objectives: We evaluated the expression of CysLTRs on eosinophil granule membranes and their functional roles in eliciting protein secretion from within eosinophil granules.

Methods: We studied secretory responses of human eosinophil granules isolated by subcellular fractionation. Granules were stimulated with cys-LTs and eosinophil cationic protein (ECP) and cytokines were measured in supernatants by ELISA and a cytokine multiplex assay, respectively. Receptor expression on granule membranes and eosinophils was evaluated by flow cytometry and western blot.

Results: The ligand-binding amino-terminal domains for both $CysLT_1R$ and $CysLT_2R$ were expressed on granule surface membranes. After cys-LT stimulation, granules secreted ECP, but not cytokines. Granule ECP secretion in response to LTC_4, LTD_4 and even LTE_4 was inhibited by montelukast, suggesting that there are other montelukast-inhibitable mechanisms, besides CysLT1R, mediating Cys-LT-elicited ECP secretion from granules. Cys-LTs, especially LTE_4 can act as agonists at P2Y12 receptor (P2Y12R) (BBRC., 337:281, 2005). MRS 2395, a P2Y12R antagonist, dose-dependently inhibited ECP release induced by cys-LTs, suggesting a role for this purinergic receptor in cys-LT-mediated granule secretion. In confirmation, we demonstrated P2Y12R membrane expression on eosinophils and isolated granules.

Conclusion: Our findings extend the recognition that cell-free eosinophil granules are secretion competent organelles. Granules respond to cys-LTs, including extracellularly formed LTE_4, via membrane-expressed receptors that elicit granule protein secretion. Thus, secretion from eosinophil granules can be initiated by receptor-mediated mechanisms elicited by intracellular and extracellular Cys-LTs.

Supported by NIH grants AI020241, AI022571 and AI051645.

(AHR) AND LUNG INFLAMMATION IN THE ABSENCE OF LYMPHOCYTES

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Background: Eosinophils are key players in Th2-driven pathologies such as allergic lung inflammation. Following IL-5- and eotaxin-mediated tissue recruitment, they release several cytotoxic and inflammatory mediators. However, their exact contribution to asthma remains controversial.

Objectives: Ability of eosinophils to promote airway hyperresponsiveness (AHR) and lung inflammation, independently of lymphocytes was investigated.

Methods: Adoptive transfers of resting or activated eosinophils from IL-5 transgenic mice were performed into naïve Balb/c, SCID and IFN-γ-deficient recipients.

Results: Adoptively transferred eosinophils induced lung inflammation, fibrosis, collagen deposition and AHR, not only in Balb/c but also in SCID recipient mice. Surprisingly, IFN-γ expression was increased in lungs from eosinophil-transferred animals. Furthermore, IFN-γ neutralization in recipients partially inhibited eosinophil-induced AHR. Moreover, IFN-γ-deficient$^-$ eosinophils or eosinophils treated with a blocking an anti-IFN-γR antibody failed to induce AHR in IFN-γ-deficient recipients. Finally, *in vitro*, at low concentrations, IFN-γ increased eosinophil peroxidase release, potentiated chemotaxis and prolonged survival suggesting the existence of an autocrine mechanism.

Conclusions: These results evidence the important and previously unsuspected contribution of eosinophils to lung inflammation, independently of lymphocytes, through production of IFN-γ, the prototypical Th1 cytokine.

This project was funded by Agence Nationale pour la Recherche

KEY ROLE OF INTERLEUKIN-15 IN THE PATHOGENESIS OF EOSINOPHILIC ESOPHAGITIS

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Background: IL-15, a cytokine that uses the common g chain, is involved in the inflammatory responses in various infectious and autoimmune diseases. Using global quantitative microarray analysis, we previously reported increased expression of IL-15 mRNA in eosinophilic esophagitis (EE) patients compared to normal individuals.

Objective: To understand the role of IL-15 in the pathogenesis of EE.

Method: The quantitative PCR analysis was performed to examine IL-15 and eotaxins gene expression in the patient esophageal biopsies and cultured esophageal epithelial cells. Further, to understand the *in vivo* role of IL-15, an experimental EE was induced in mice and esophageal eosinophilia was examined by performing anti-MBP immunostaining analysis.

Results: Our quantitative PCR analysis demonstrated a 4-5-fold increase in IL-15 mRNA expression in the esophagus of EE patients compared to normal individuals. In addition, levels of esophageal IL-15 mRNA correlated ($p<0.001$) with esophageal eosinophils. Furthermore, to understand *in vivo* role of IL-15, we delivered recombinant murine IL-15 intra-nasally to naïve mice and found a dose-dependent accumulation of eosinophils in the esophagus. Interestingly, mice genetically deficient in IL-15rα were protected from allergen-induced esophageal eosinophilia compared to the wild type mice; whereas, a comparable airway eosinophilia was observed in the same mice. In particular, esophageal eosinophils levels in allergen challenged IL-15 gene-deficient mice were $3.7 \pm 0.3/mm^2$ compared with $31.5 \pm 12.6/mm^2$ in wild type mice (compared with $1.4 \pm 0.4/mm^2$ and $1.7 \pm 0.3/mm^2$ in saline exposed mice, respectively). To further understand the mechanism accounting for IL-15 induced experimental EE, we treated primary esophageal epithelial cells (from mice and man) with IL-15. Interestingly, a dose dependent increase in the expression of eotaxin-1 and eotaxin-3 mRNA was observed. Additionally, to confirm that eotaxin has a role in IL-15-induced esophageal eosinophilia, wild type and eotaxin-deficient mice were exposed to recombinant IL-15. A 3-fold reduced ($P < 0.002$) level of esophageal eosinophilia in eotaxin-1 gene-deficient mice compared to wild type mice was observed.

Conclusion: These studies demonstrate that IL-15, via an eotaxin-dependent pathway, is both sufficient and required for allergen-induced esophageal eosinophilia. Together with our human expression data, we propose an essential role for IL-15 in the pathogenesis of EE.

This project was funded by a grant from National Institute of Health (NIH).

INTERACTIONS BETWEEN EOSINOPHILS AND NERVES IN ATOPIC DERMATITIS

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Background: Atopic dermatitis (AD) is a disease characterized by an increased density of nerve terminals in the skin, and a large number of circulating eosinophils. Extracellular eosinophil products, indicating eosinophil activation, are present at high levels in AD lesions. The extreme itch sensation in AD is transmitted by sensory neurons whose cell bodies are located in dorsal root ganglia (DRG). We investigated whether eosinophils interacted with sensory neurons in skin from humans with AD, in a mouse model of AD, and in 24 hour co-cultures.

Objectives: To determine the extent of interaction between sensory neurons and eosinophils in atopic dermatitis, and to characterize the effects on cutaneous nerves.

Methods: Using antibodies to the nerve-specific protein PGP9.5 and to eosinophil major basic protein (MBP), we detected eosinophil products and nerves in normal and lesional atopic human skin. Cultures of DRG sensory neurons from mice were immunostained with antibodies against eosinophil chemotactic factors and adhesion molecules. Mouse eosinophils were isolated and co-cultured with DRG sensory neurons for 24 hours, and the changes in neuronal morphology were quantified by measurement of neurite extension and branching. A mouse model of AD was established by tape stripping the epidermis and sensitizing mice epicutaneously to antigen, and skin sections from mice were immunostained for MBP and PGP9.5.

Results: We found that 20-60% of nerves are physically associated with MBP in human lesional atopic skin, while normal skin contains little or no MBP. Cultured DRG neurons from mice produce eotaxin-1, a chemotactic factor for eosinophils, as well as the adhesion molecules ICAM-1 and VCAM-1. The expression of ICAM-1 and VCAM-1 by DRG was prevented with a blocking antibody to nerve growth factor (NGF). When cultured with mouse eosinophils for 24 hours, DRG neurons bind eosinophils, indicating functional recruitment and adhesion. The interaction of sensory neurons and eosinophils over 24 hours dramatically altered the morphology of DRG, causing increased branching and longer neurites. This effect did not require contact between cells and was not blocked by an antibody to NGF. Neurite extension and branching did not occur when neurons were cultured with mast cells. In the mouse model of AD, there was a significant increase in nerves in the skin of sensitized mice, similar to the increase in nerve number and length in human atopic skin.

Conclusions: Eosinophils and nerves interact in the skin of atopic dermatitis patients. This interaction may mediate nerve growth and contribute to the increased sensation of itch in AD.

This project was funded by a grant from the National Institutes of Health to the Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland Oregon.

A KEY ROLE FOR EOTAXIN-1 IN ANTIFILARIAL IMMUNITY VIA REGULATION OF EOSINOPHIL ACTIVATION

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Background: Ocular Onchocerciasis is caused by the filarial nematode *Onchocerca volvulus*. Microfilariae migrate through subcutaneous tissues of infected individuals and can penetrate the eye where they cause inflammatory responses leading to visual impairment and blindness. Inflammatory responses are characterized by the infiltration of neutrophils and eosinophils into the corneal stroma.

Objectives: This study examined the role of Eotaxin-1 in the migration of eosinophils and survival of filarial worms in a mouse model of Onchocerciasis. In addition, we determined Eotaxin-1 dependent eosinophil activation by filarial proteins.

Methods: We infected Balb/c mice with the filaria *Litomosoides sigmodontis*. Adult worms migrate to the pleural cavity rather than the eye. Additionally, mice were injected sc with protein extracts from *L. sigmodontis*, followed by injection of *L. sigmodontis* extract in the cornea.
Systemic immune responses were measured by splenic cytokine production and antibody levels in the serum by ELISA. For the infection model, we then determined worm load in and eosinophil migration to the pleural cavity by microscopy and FACS analysis, respectively. For the immunization model, we measured neutrophil and eosinophil migration to the corneal stroma by immunohistochemistry.
For *in vitro* analysis of eosinophil activation, eosinophils were elicited by sc injection of Ova, followed by ip challenge 2 days prior to ip lavage. Eosinophils were purified by magnetic bead sorting and stimulated with filarial proteins. Activation was determined by expression of surface activation markers MHCII, CD69 and CD86 as well as cytokine production (IL-6, MIP-2).

Results: We found that in both models, antibody production (IgG1 and IgG2a) was decreased in Eotaxin-1-/- mice compared to wildtype Balb/c mice. IFN-□ production was increased in Eotaxin-1-/- mice (both models) while IL-5 levels were similar. Eotaxin-1 was absent from the pleural cavity of Eotaxin-1-/- mice. Surprisingly, this was not reflected by a reduced eosinophil infiltration to the pleural cavity in the infection model. Although eosinophil numbers were comparable, worm load was increased in Eotaxin-1-/- mice. In the immunization model, neutrophil migration into the corneal stroma was increased in Eotaxin-1-/- mice. Following *in vitro* stimulation, surface expression of MHCII, CD69 and CD86 was upregulated. Eosinophils produced increased levels of IL-6 and MIP-2. The increased cytokine production was absent in eosinophils from Eotaxin-1-/- mice.

Conclusion: Surprisingly, we found Eotaxin-1 dispensable for migration of eosinophils to the site of infection/inflammation and expect compensation of the lack of Eotaxin-1 by Eotaxin-2 or RANTES. However, our studies demonstrated a clear role for Eotaxin-1 in eosinophil activation *in vitro* and control of filarial worm loads *in vivo*.

CD34 IS REQUIRED FOR THE INFILTRATION OF INFLAMMATORY CELLS INTO THE MOUSE COLON DURING DSS-INDUCED COLITIS

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Background: Eosinophil infiltration of gut tissue plays a key role in the pathogenesis of inflammatory bowel diseases (IBD), such as ulcerative colitis. Using a model of allergic asthma, we previously demonstrated that eosinophil migration requires surface expression of the sialomucin CD34, and that *Cd34* deletion dampens asthmatic responses in mice.

Objectives: Since CD34 is critical for eosinophil migration, we investigated a role for CD34 in the migration of inflammatory cells into the colon using a mouse model of IBD.

Methods: To induce ulcerative colitis, we treated animals with 3.5% dextran sodium sulfate (DSS) and monitored the appearance of clinical symptoms including weight loss, rectal bleeding and diarrhea. Mice were sacrificed after eight days of treatment and we measured colon length, enumerated hematopoietic lineage subsets infiltrating gut tissue by flow cytometry and prepared colon sections for histology to determine the severity of gut pathology. In order to determine the significance of CD34 expression on hematopoietic cells in the development and progression of IBD, we reconstituted wild type mice with *Cd34*$^{-/-}$ bone marrow to generate chimeras.

Results: We found that*Cd34*$^{-/-}$ mice are highly resistant to DSS-induced IBD with significantly less weight loss and colon shortening than wildtype controls. Histological analysis of *Cd34*$^{-/-}$ colons revealed less crypt loss and tissue infiltrate, and overall reduced disease severity. We found that approximately 40% of the infiltrating blood cells are eosinophils and peripheral eosinophil levels are reduced following disease induction. Intriguingly, eosinophils harvested from the colon express high levels of CD34 and represent the majority of CD34^{+} cells within inflamed gut tissue. Protection from DSS-induced IBD is largely recapitulated in mice reconstituted with *Cd34*$^{-/-}$ bone marrow, demonstrating the requirement for CD34 expression on hematopoietic cells in mucosal inflammation.

Conclusions: Our findings demonstrate a key role for CD34 on hematopoietic cells in the pathology of ulcerative colitis. Gut eosinophils express high levels of CD34 and, similar to our findings in allergic asthma, we demonstrated that CD34 is required for optimal eosinophil migration *in vivo* and *Cd34* deletion results in decreased gut inflammation during IBD. Taken together, our findings highlight CD34 as a potential therapeutic target for IBD treatment.

The research was funded by the Allergen Network Centre of Excellence (3.14). SM and MRH hold CIHR and Hearth & Stroke Transfusion Science Fellowships from the Centre for Blood Research (UBC). KMM is a Michael Smith Foundation Scholar (Senior) and CBR Member.

RESPONSE TO MEPOLIZUMAB IN PATIENTS WITH T CELL MEDIATED HES

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Background: Lymphocytic variant hypereosinophilic syndrome (L-HES) is characterized by sustained IL-5 production by T cells, accounting for eosinophil expansion. Previous studies have indicated that increased serum TARC, a chemokine known to be induced by IL-4/-13, may be a diagnostic biomarker for this variant. Patients with L-HES generally respond well to corticosteroid (CS) therapy, but require long-term treatment. The monoclonal anti-IL-5 Ab, mepolizumab (MEPO), has recently been proven an effective CS-sparing agent in patients with HES.

Objectives: Given the marked overproduction of IL-5 by T cells in L-HES, and the possibility that some disease manifestations may be due to other mediators produced by the deregulated T cells, we investigated whether these patients respond to MEPO to the same extent as other patients enrolled in the MHE100185 trial.

Methods: Evidence for L-HES was searched using flow cytometry, PCR analysis of TCR gene rearrangements, and measurement of serum TARC levels. Clinical data including serial eosinophil levels and CS doses during the trial, study treatment arm, and achievement of primary, secondary and post-hoc study endpoints was provided by GSK.

Results: Among 70 patients for whom data on T cell phenotype and/or clonality was available, 18 (26%) showed evidence of an underlying T cell disorder (L-HES), 9 of whom had CD3-CD4+ T cells. Eleven patients with T cell abnormalities received MEPO, and 3 failed to reach the primary endpoint, vs 2/24 patients with normal T cell studies (p=0,30). Two non-responders in the L-HES group were ultimately diagnosed with T cell lymphoma. A higher proportion of patients with a normal T cell profile were able to maintain eosinophil levels below 600/μl with MEPO alone compared to those with T cell-mediated disease (96% vs 63,6%, p=0,026). Requirement for a CS maintenance dose was observed in 7/11 vs 10/24 patients with vs without T cell disease (p=0,29). We also studied the relationship between serum TARC levels and response to MEPO. Among 82 patients for whom serum TARC levels were available, an increase >1500 pg/ml was observed in 24, including 14 of the 18 L-HES patients. Among patients under MEPO, prolonged CS withdrawal was achieved in 3/12 vs 15/29 of those with high vs normal TARC (p=0,17), and eosinophils remained <600/μl in 7/12 (58%) vs 25/29 (86%) (p=0,09). In the placebo arm, those with TARC>1500 were less likely to reach the primary endpoint (PDN ≤10 mg for ≥8 weeks) (p=0,07).

Conclusions: Patients with L-HES benefit from MEPO in terms of CS dosing, but eosinophil depletion is less marked under MEPO alone than in patients with a normal T cell profile. Increased serum TARC is associated with a lower placebo response rate. These observations indicate that eosinophil expanion is the result of a strong and permanent cytokine-driven process in such patients. Follow-up in the open label extension study should determine whether disease control under MEPO requires more frequent infusions and/or a maintenance CS dose in this patient subgroup. Finally, special attention should be paid to the possible existence of unrecognized T cell lymphoma in patients with T cell-driven hypereosinophilia.

This project was funded by a grant from the Fonds National de la Recherche Scientifique, the National Institutes of Health, and received logistical and financial support from GlaxoSmithKline.

DOWNSTREAM EFFECTORS OF FIP1L1-PDGFRα AS TARGETS FOR THERAPY IN CHRONIC EOSINOPHILIC LEUKEMIA

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Background: Chronic eosinophilic leukemia (CEL) is a rare hematological malignancy characterized by overproduction of eosinophils and is frequently caused by the *FIP1L1-PDGFRA* fusion gene. The small molecule kinase inhibitor imatinib is very successful for the treatment of FIP1L1-PDGFRα positive CEL, but requires lifelong treatment of the patients.

Objectives: This study aimed at the identification of specific downstream effectors of FIP1L1-PDGFRα that could potentially be used as additional therapeutic targets and/or diagnostic markers in CEL.

Methods: Gene expression patterns of untreated and kinase inhibitor treated human FIP1L1-PDGFRα positive EOL-1 cells were compared. The importance of the identified genes for the proliferation and survival of EOL-1 cells was further explored *in vitro* using an RNAi approach.

Results: We identified 51 genes to be differentially expressed upon treatment of EOL-1 cells with both imatinib and sorafenib. We selected 14 significantly up- or downregulated genes and demonstrated that they were modulated to a similar extend by BCR-ABL1 and FLT3 kinase activity as detected in the context of FIP1L1-PDGFRα. One of the genes upregulated by FIP1L1-PDGFRα kinase activity is CCL2, a member of the CC□ subfamily of chemotactic cytokines with a known role in cancer cell proliferation. CCL2 was 100-fold upregulated downstream of FIP1L1-PDGFRα. siRNA mediated knock down of *CCL2* expression significantly decreased the proliferation of EOL-1 cells. The antiproliferative effect on EOL-1 cells was strongly increased when we combined CCL2 knock down with a low nanomolar dose of imatinib. Addition of CCL2 to the growth medium abolished the inhibitory effect of the siRNA, indicating that CCL2 production downstream of FIP1L1-PDGFRA acts through an autocrine loop.

Conclusions: Our results indicate that CCL2 production in EOL-1 cells is upregulated by FIP1L1-PDGFRα kinase activity and that CCL2 stimulates the proliferation of EOL-1 in an autocrine way. CCL2 may represent a novel target for therapy in CEL. Further research is currently ongoing to elucidate the precise role of CCL2 in EOL-1 cell proliferation and its function downstream of FIP1L1-PDGFRα.

POSTERS: SESSION I

Regulation of eosinophil development, functional maturation, and death (Posters 1-4)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
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POSTER #1

HUMAN C/EBPe ISOFORMS DIFFERENTIALLY REPROGRAM EOSINOPHIL LINEAGE COMMITMENT, GENE EXPRESSION AND TERMINAL DIFFERENTIATION

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Background: Human C/EBPε is required for differentiation of eosinophils and neutrophils and is expressed as four distinct isoforms (32, 30, 27, 14kD) through alternative usage of promoters, translational start sites and alternative RNA splicing. C/EBPε$^{32/30}$ function as transcriptional activators. C/EBPε^{27} represses GATA-1 transactivation of eosinophil genes. C/EBPε^{14}, which lacks a transactivation domain, may function as a dominant negative regulator and repress the activities of C/EBPε$^{32/30}$ or other C/EBP members.

Objectives: The objective was to study the activities of the C/EBPε isoforms in cord blood CD34+ progenitors to define their roles in eosinophil development.

Methods: We assessed mRNA expression by RT-PCR and protein expression by Western blotting for the C/EBPε isoforms during IL-5 induced eosinophil differentiation of CD34+ progenitors. An MSCV-based bicistronic retroviral vector (pGCDNsam IRES-EGFP) was used to ectopically over-express the C/EBPε isoforms in transduced CD34+ progenitors. CD34+/GFP+ cells were sorted by FACS, colony forming cell assays performed using multi-lineage or lineage-specific cytokines to drive eosinophil, neutrophil, or erythroid development, or cells cultured in suspension with IL-5 to drive eosinophil differentiation. Colonies were enumerated using histochemical and enzyme staining to differentiate eosinophil, neutrophil, granulocyte-macrophage and erythroid colonies.

Results: The C/EBPe isoforms are differentially expressed in a temporally regulated manner during eosinophil differentiation, starting with C/EBPe$^{32/30}$ and e^{14}, followed by e^{27}. Progenitors transduced with C/EBPε$^{32/30}$ default exclusively to eosinophil differentiation and gene expression, independent of IL-5, and regardless of including cytokines to specifically induce other lineages. The C/EBPε^{27} isoform strongly inhibits eosinophil differentiation and gene expression, including GATA-1, promoting granulocyte-macrophage differentiation. The C/EBPε^{14} isoform acts as a dominant negative regulator, strongly inhibiting eosinophil development and gene expression, driving cells to erythroid development, likely by inhibiting the activity of C/EBPe$^{32/30}$ or other C/EBP family members such as C/EBPa that are required for myeloid development.

Conclusions: The C/EBPε isoforms are differentially expressed during eosinophil development, instruct stem cell development towards or away from the eosinophil lineage consistent with their activator vs. repressor activities and interactions with transcription factors required for eosinophil development (GATA-1), and likely have combinatorial roles in fine-tuning eosinophil gene transcription and terminal differentiation.

This project was funded by NIH grant AI033043 to SJA. RJB was funded in part by NIH Training Grant T32DK007739.

POSTER #2

DIFFERENTIATION OF EOSINOPHILS FROM UNSELECTED MOUSE BONE MARROW

Kimberly D. Dyer[1], Jennifer M. Moser[1], Meggan Czapiga[2], Steven J. Siegel[1], Caroline M. Percopo[1], Elizabeth R. Fischer[3] Paige Lacy[4], Redwan Moqbel[5] and Helene F. Rosenberg[1]

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Background: Research into the function and the mechanisms controlling eosinophils has been hampered by the difficulty of collecting cells in sufficient number and purity to perform carefully controlled experiments. Thus far, *in vitro* differentiation models have failed to achieve simultaneous high purity and sufficient cell number.

Objective: Devise an *ex vivo* culture system that will support the differentiation of eosinophils from mouse bone marrow with the intent to achieve a large number of eosinophils at high purity that can be used for developmental and functional studies.

Methods: Bone marrow cells were collected, red blood cells were lysed and the bone marrow cells were cultured at 10^6/mL in a base media supplemented with 100 ng/mL stem-cell factor and 100 ng/mL FLT3-Ligand from day 0 to day 4. On day 4, the medium containing SCF and FLT3-L was replaced with medium containing 10 ng/mL mouse interleukin-5 only. On day 8, the cells were moved to new flasks and maintained in fresh medium supplemented with rmIL-5. Every other day, from this point forward, one-half of the medium was replaced with fresh medium containing rmIL-5 and the concentration of the cells was adjusted each time to 10^6 /mL.

Results: Starting with 3 x 10^6 bone marrow progenitors, we typically obtain >10^7 eosinophils (90-100% purity, 99% viability) by day 10-12 of culture. The bone-marrow derived eosinophils (bmEos) express immunoreactive major basic protein, Siglec F, IL-5 receptor alpha chain, 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. Electron micrographs of bmEos (day 10 of culture) reveal eosinophils with apparently normal morphology, including a polymorphic nucleus and granules with lucent cores. BmEos undergo chemotaxis toward mouse eotaxin-1, degranulate in response to platelet activating factor and produce characteristic cytokines.

Conclusions: We have devised a method that optimizes both yield and purity of phenotypically and functionally mature eosinophils from unselected mouse bone marrow progenitors.

POSTER #3

CD 30 INDUCED HUMAN EOSINOPHIL APOPTOSIS IS MEDIATED BY INTRINSIC PATHWAY OF CASPASE CASCADE

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Background: Inhibition of eosinophil apoptosis has been suggested as a key role in prolonging allergic inflammation.CD 30(Ki-1 antigen), a member of the TNFR family, was originally identified on primary and cultured Hodgkin and Reed Stenberg cells of Hodgkin's disease. CD30 is chiefly expressed on the surface of activated T cells, NK cells, and lymphocytes infected with HIV or EBV. Eosinophils also expressCD30 molecules weakly and the presentation is up-regulated in the cells undergoing apoptosis.

Objectives: We tried to determine whether CD30 molecule have a role in inducing apoptosis of human apoptosis of human peripheral blood eosinophils, and to investigate the signaling pathways of the apoptosis through CD 30.

Methods: We drew 90mL of peripheral blood from healthy or mild allergic donors, and purified eosinophils using MACS system at Uijeongbu St Mary's Hospital. Eosinophils were inculated in 10% FBS with or without IL-5, dexamethasone, or cultured in pretreated wells with anti-CD30 (BerH8). We measured the rates of apoptosis during the time interval using flow cytometry. Addtionally, to elucidate the mechanisms of apoptosis, we measured the amount of caspase 3, 9, and bcl-2 protein by western blot. After eosinophiles were cultured in pretreated wells with caspase 9 inhibitor, caspases 3 inhibitor 200 μM and anti-CD30, we measured apoptosis rate using flow cytometry.

Results: Ligation of CD30 induced intense apoptosis of eosinophils, which was faster than dexamethasone. Activation of CD30 molecule on eosinophils by anti-CD 30 antibody decreased the expression of pro-caspase 3 and 9, and bcl-2 as well. And the ratio of apoptosis of eosinophils cultured with caspases 9 inhibitor, anti CD-30 antibody was lower than that of cultured with anti CD-30 antibody.

Conclusions: Anti-CD30 antibody could intensively accelerate the apoptosis of human peripheral blood eosinophils. And the apoptotic effect of the antibody was more rapid than that of dexamethasone. Activation of CD30 molecule on eosinophils decreases the expression of pro-caspase 3 and 9, and bcl-2 as well. The apoptotic effect of anti-CD30 antibody is mediated through the activation of caspase cascade and bcl-2 protein which is involved in the process of the apoptosis. Caspase 9 inhibitor can decrease the apoptosis induced by anti-CD30 antibody.

POSTER #4

IS TMEM103, A NOVEL PUTATIVE PROTEIN, EXPRESSED DURING EOSINOPHIL DEVELOPMENT?

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Background: The myriad of eosinophil related diseases has led us to investigate the development of eosinophils from hematopoietic stem cells in an attempt to understand the signals prompting eosinophil production in the bone marrow. Gene profiling of developing eosinophils has shown that a novel hypothetical protein, TMEM103 is expressed as an mRNA during eosinophilopoiesis.

Objective: To determine if the putative TMEM103 protein is expressed in developing eosinophils and to elucidate its function.

Methods: Real-time quantitiative RT-PCR was done on a LightCycler with RNA from $CD34^+$ cord blood cells stimulated with IL-5. Sucrose gradients for separation and RT-PCR for quantitation was used to determne that TMEM103 transcripts were associated with developing ribosomes. Polyclonal antibodies were raised to recombinantTMEM103 and monoclonal antibodies are currently being developed against rTMEM103.

Results: TMEM103 mRNA is expressed in IL-5 stimulated $CD34^+$ cells, bone marrow and in mature eosinophils. The transcript, TMEM103, has been shown to be associated with ribosomes and the protein has been identified by western blot analysis using polyclonal antibodies in developing eosinophils. Furthermore, conservation of TMEM103 exons is high in the mouse.

Conclusions: TMEM103 protein is likely expressed in the developing eosinophils. To confirm this expression monoclonal antibodies will be used for immunoprecipitation of TMEM103 in an invitro model of developing eosinophils. Mass spectrometry will be used to identify the precipitated protein.

POSTERS: SESSION II

Eosinophil functions (trafficking, activation, and signaling) (Posters 5-14)

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6	**Eriksson, Jenny**	**POST-TRANSLATIONAL GLYCOSYLATION DETERMINES THE CYTOTOXIC CAPACITY OF EOSINOPHIL CATIONIC PROTEIN (ECP)**	**Jenny Eriksson and Per Venge**
7	**Jönsson, Ulla-Britt**	**ASSOCIATIONS OF ECP (EOSINOPHIL CATIONIC PROTEIN)-GENE POLYMORPHISMS TO ALLERGY, ASTHMA, SMOKE HABITS AND LUNG FUNCTION IN TWO ESTONIAN AND SWEDISH SUB COHORTS OF THE ECRHS II STUDY**	**Ulla-Britt Jönsson, Lena Douhan Håkansson, Rain Jõgi, Christer Janson, Per Venge**
8	**Johansson, Mats**	**EOSINOPHIL β1 INTEGRIN ACTIVATION: A POTENTIAL PHENOTYPIC MARKER FOR THE HOSPITAL-PRONE ASTHMATIC PATIENT**	**Mats W. Johansson, William W. Busse and Deane F. Mosher**
9	**Mathur, Sameer**	**DIRECT ACTIVATION OF EOSINOPHIL DEGRANULATION BY THYMIC STROMAL LYMPHOPOIETIN (TSLP)**	**Ellen B. Cook, James L. Stahl, Elizabeth A. Schwantes, Cynthia J. Koziol-White, Paul J. Bertics, Frank M. Graziano and Sameer K. Mathur**
10	**Morgan, Paul**	**INTRACELLULAR INHIBITION OF EOSINOPHIL PEROXIDASE-CATALYZED OXIDATION**	**Karen Page, Larry Mason, Marion Kasaian, Xiaoke Yang, and Paul Morgan**
11	**Nissim Ben Effraim, Alon**	**EFFECTS OF TISSUE HYPOXIA ON HUMAN EOSINOPHILS**	**Alon H. Nissim Ben Efraim, Ron Eliashar, Francesca Levi-Schaffer**
12	**Percopo, Caroline**	**EOSINOPHIL DEGRANULATION IS INHIBITED BY INTERACTION WITH RESPIRATORY SYNCYTIAL VIRUS**	**Caroline M. Percopo, Kimberly D. Dyer and Helene F. Rosenberg**
13	**Shamri, Revital**	**EOTAXIN-MEDIATED MOUSE EOSINOPHIL DEGRANULATION AND SECRETION OF EOSINOPHIL-ASSOCIATED RNASES**	**Revital Shamri, Rossana C.N. Melo, and Peter F. Weller**
14	**Spencer, Lisa**	**NOTCH SIGNALING UNDERLIES FULL AND SUSTAINED EOSINOPHIL ACTIVITIES**	**Amy L. Radke, Lauren L. Reynolds, Peter F. Weller and Lisa A. Spencer**

POSTER #5

POLYMORPHISM IN THE EOSINOPHIL PROTEIN X / EOSINOPHIL DERIVED NEUROTOXIN GENE IS LINKED TO VISCERAL LEISHMANIASIS.

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Background: Kala-azar or Visceral leishmaniasis (VL), is the most severe form of leishmaniasis. In Sudan about 50% of the patients develop post-kala-azar dermal leishmaniasis (PKDL) after successful treatment against VL and recent findings indicate eosinophil involvement in the lesions. The EPX/EDN405(G>C) gene polymorphism is closely linked to the eosinophil cellular content of EPX/EDN.

Objectives: To investigate possible linkages between genetic variations in the EPX/EDN gene and endemic exposure to VL.

Methods: The intron polymorphism at position 405 in the EPX/EDN gene (G>C rs2013109) was analysed in 239 samples from a Sudanese population, (64 healthy controls, 100 VL and 73 PKDL samples). As a reference 300 healthy controls from Sweden were analysed. The samples were analysed using real time-PCR with TaqMan® reagents.

Results: No significant difference was seen between the control groups from Sweden and Sudan as to the prevalence of the EPX/EDN405(G>C) genotypes (GG 66%, GC 28%, CC 6%). In the cohort infected by *Leishmania Donovani* (n=173) we found significant differences in genotype prevalences (GG 89%, GC 10%, CC 1%) when compared to the healthy controls (p=0.0001). In the subgroup affected by PKDL the genotype distribution was different from the VL subgroup (GG 96%, GC 3%, CC 1% vs GG 84%, GC 15%, CC 1%) (p=0,03). In all cases the G-allele of the EPX/EDN405(G>C) gene polymorphism was the predominant with a distribution of PKDL>VL>healthy controls.

Conclusions: There is an over expression of the EPX/EDN405(G>C) G-allele in the Sudanese population suffering from VL. Out of the treated patients an increased frequency of G-allele was found in the population developing PKDL. Our findings suggest that the eosinophil content of EPX/EDN may be reduced in subjects with VL and PKDL, which may alter the susceptibility and reactions to infections by the parasite *Leishmania Donovani.*

POSTER #6

POST-TRANSLATIONAL GLYCOSYLATION DETERMINES THE CYTOTOXIC CAPACITY OF EOSINOPHIL CATIONIC PROTEIN (ECP)

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Background: Native Eosinophil Cationic Protein (ECP) is a heterogeneous protein; the molecular weight can differ from 15-22 kDa depending on the degree of glycosylation of the molecule. Previous studies have shown that only a fraction of ECP stored in the granules is biologically active in terms of cytotoxicity. In general, higher molecular weight ECP is less cytotoxic in vitro. Posttranslational modifications in terms of glycosylation might therefore determine the cytotoxic activity of ECP.

Objectives: To examine whether removal of carbohydrates would enhance the cytotoxic activity of ECP or even induce activity of inactive forms.

Methods: The cytotoxic activity of high molecular weight ECP purified from healthy blood donors was tested against the small cell lung cancer cell line NCI-H69 using the FMCA assay, before and after enzymatic deglycosylation. ECP from subjects carrying the ECP 434GG genotype was selected for the study. The variants of ECP were also analyzed by SELDI-TOF MS to monitor the changes in molecular mass of the protein after deglycosylation.

Results: Five high molecular weight pools of ECP (HMW-ECP I to V) with various degree of glycosylation were tested regarding their cytotoxic activity at concentrations ranging from 0.036 μM to 0.57 μM. The cytotoxic activity ranged from IC50 values of >0.57 μM to 0.14 μM; the lowest activity was associated to ECP pools containing high molecular weight ECP (HMW-ECP I), and the highest activity was seen in the pool containing ECP of lowest molecular weight (HMW-ECP V).
After deglycosylation with the N-Glycosidase F enzyme, the molecular masses of ECP species in pools HMW-ECP I – III were reduced to the same molecular mass of 15.77 kDa and acquired potent and similar cytotoxic activities. The masses and activities of HMW-ECP IV and V were unaffected by enzymatic treatment.

Conclusions: The posttranslational modifications of ECP have a major impact on the cytotoxic activity of the molecule, a large fraction of native and potentially cytotoxic ECP seems to be stored in the eosinophil granules in a non-cytotoxic, highly glycosylated

POSTER #7

ASSOCIATIONS OF ECP (EOSINOPHIL CATIONIC PROTEIN)-GENE POLYMORPHISMS TO ALLERGY, ASTHMA, SMOKE HABITS AND LUNG FUNCTION IN TWO ESTONIAN AND SWEDISH SUB COHORTS OF THE ECRHS II STUDY

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Background: The Eosinophil Cationic Protein (ECP) is a potent multifunctional protein. Three common polymorphisms are found in the ECP gene, which determine the function and production of the protein.

Objectives: To study the relationship of the three common ECP gene polymorphisms to signs and symptoms of allergy and asthma in a community based cohort.

Methods: Swedish and Estonian subjects (n=767) were randomly selected from the larger cohort of the ECRHS II study cohort. The prevalence of the gene polymorphisms ECP434(G>C) rs2073342, ECP562(G>C) rs2233860, and ECP c.-38(A>C) rs2233859 were analysed by DNA sequencing performed on Beckman Coulter CEQ™ 2000 and 8000 DNA analysis systems, and/or by the TaqMan 5' nuclease allelic discrimination assay, and related to questionnaire-based information of allergy, asthma, smoking habits and to lung functions.

Results: Genotype prevalences showed both ethnic and gender differences. Close associations were found between the ECP434(G>C) genotypes and smoking habits, lung function and expression of allergic symptoms. In males the ECP c.-38(A>C) genotypes were associated to the subject being atopic. Non-allergic asthma was predominantly found in women carrying the 434GG genotype. The ECP562(G>C) showed associations to smoking habits.

Conclusions: Our results show intriguing associations of symptoms of allergy and asthma to ECP-genotypes suggesting important roles of this protein in these disease processes. ECP may be involved in impairment of lung functions in disease.

This project was funded by grants from The Swedish Medical Research Council, The Swedish Heart and Lung Foundation and The Asthma and Allergy Research Foundation

POSTER #8

EOSINOPHIL β1 INTEGRIN ACTIVATION: A POTENTIAL PHENOTYPIC MARKER FOR THE HOSPITAL-PRONE ASTHMATIC PATIENT

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Background: In a pilot study of asthma patients, β1 integrin activation on blood eosinophils, reported by the monoclonal antibody N29, was inversely correlated with the forced expiratory volume in 1 s (FEV_1).

Objectives: To determine the relationship between N29 epitope expression on peripheral blood eosinophils and phenotypic features of asthma in a population of patients with disease of varying severity.

Methods: Peripheral blood eosinophils were obtained from subjects (severe and non-severe asthmatics and normal donors) enrolled in the Severe Asthma Research Program (SARP) and assayed by flow cytometry.

Results: N29 reactivity was higher in asthma than in normals, but there was no significant difference between severe and non-severe asthmatic groups. However, N29 reactivity was higher in subjects who had been hospitalized for asthma exacerbations. Interestingly, we also found that N29 reactivity was higher in subjects receiving low to medium doses of inhaled corticosteroids (ICS), whereas those on high doses of ICS had lower expression of the N29 epitope marker.

Conclusions: Eosinophil β1 integrin activation state does not appear to discriminate between severe and non-severe asthma, but rather may be a marker of an important asthma phenotype – asthma patients who have required hospitalization. As a history of previous hospitalization is known to be a risk factor for more severe disease and subsequent hospitalizations, our results indicate that activation state of eosinophil β1 integrin is a biomarker of this at-risk asthma patient. Furthermore, we have preliminary evidence that expression of the N29 epitope is associated with lower to medium doses of ICS, thus indicating that more aggressive treatment with high doses of ICS may reduce integrin activation and provide, through mechanisms unknown, protection from hospitalization. Therefore, our data raise the possibility that the N29 epitope is a biomarker for an asthma phenotype that puts patients at risk for severe exacerbations, and monitoring N29 epitope expression during treatment with ICS may be helpful in reducing this future risk of asthma.

This project was funded by grants P50 HL56396, R01 HL69116, P01 HL88594, M01 RR03186, and UL1 RR25011 from the National Institutes of Health and by Robert Draper Technology Funding from the Graduate School

POSTER #9

DIRECT ACTIVATION OF EOSINOPHIL DEGRANULATION BY THYMIC STROMAL LYMPHOPOIETIN (TSLP)

Ellen B. Cook[1], James L. Stahl[1], Elizabeth A. Schwantes[1], Cynthia J. Koziol-White[2], Paul J. Bertics[2], Frank M. Graziano[1] and Sameer K. Mathur[1]

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Background: A role for thymic stromal lymphopoietin (TSLP) in allergic disease was first identified when it was shown to activate $CD11c^+$ dendritic cells leading to preferential differentiation of CD4+ T cells into Th2 polarized effector cells. Thus, TSLP is considered a cytokine promoting allergic inflammation. More recently, the discoveries of additional cellular targets for TSLP, such as T cells and mast cells have suggested multiple pathways through which TSLP can influence allergic inflammation.

Objectives: Given the presence of TSLP and eosinophils as prominent components of allergic inflammation, we sought to determine whether eosinophils express the TSLP receptor α-chain (TSLPR) and respond directly to TSLP stimulation.

Methods: Eosinophil mRNA expression for TSLPR was examined by real-time quantitative PCR of purified human peripheral blood eosinophils treated with cytokines in various combinations, including IL-3, IL-5, GM-CSF, and TNFα. Immunoblotting for TSLPR protein expression was performed of eosinophil lysates with a goat anti-TSLPR antibody. Flow cytometry with a goat anti-TSLPR antibody was used to detect surface TSLPR expression. Eosinophils were stimulated with human recombinant TSLP at various concentrations for 4 hours and supernatants were analyzed by ELISA for degranulation of eosinophil derived neurotoxin (EDN).

Results: Expression of mRNA and cell surface TSLPR was upregulated by stimulation with TNFα and IL-5 family cytokines. TSLPR protein was also detectable by Western blotting of eosinophil lysates. Stimulation of eosinophils with TSLP resulted in phosphorylation of the STAT5 transcription factor. In addition, eosinophil stimulation with TSLP resulted in degranulation of eosinophil derived neurotoxin (EDN) at levels comparable to IL-5. Furthermore, the TSLP-stimulated eosinophil degranulation was inhibited by a functional blocking antibody to the TSLPR.

Conclusions: This study demonstrates a role for eosinophils as a target of the allergic inflammatory cytokine, TSLP. Thus, in allergic inflammation, multiple pathways can serve to activate eosinophils, perhaps in a synergistic manner.

This project was funded by grants from the NIH.

POSTER #10

INTRACELLULAR INHIBITION OF EOSINOPHIL PEROXIDASE-CATALYZED OXIDATION

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Background: Eosinophil peroxidase (EPO) is a major enzyme expressed by eosinophils, and is thought to contribute to oxidative stress and damage in eosinophilic diseases through its reactive products. EPO can catalytically oxidize the non-fluorescent molecule Amplex Red to generate a detectable fluorescent molecule, resorufin. Small polyphenolic compounds such as resveratrol, a natural compound found in the skin of red grapes, have been shown to irreversibly inactivate EPO peroxidation, *in vitro*.

Objectives: The aim of this research was to develop an assay that can measure the EPO peroxidation reaction within an eosinophil and to evaluate the intracellular potency of several EPO inhibitors.

Methods: A fluorescence-microscopy-based assay was developed to measure the catalytic activity of human EPO within eosinophils. Images were captured of human eosinophils treated with H_2O_2, Amplex red dye and various concentrations of small molecule inhibitors, including resorcinol and resveraterol.

Results: Eosinophils treated with hydrogen peroxide and Amplex Red clearly showed an increase in fluorescence. The observed fluorescence can be efficiently inhibited by pre-treating the cells with potent irreversible EPO inhibitors.

Conclusions: These results demonstrate a novel assay for evaluating the potency of EPO inhibitors directly within eosinophils.

POSTER #11

EFFECTS OF TISSUE HYPOXIA ON HUMAN EOSINOPHILS

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Background: Eosinophils (EOS) are involved in various inflammatory processes including allergic inflammation (AI) in which angiogenesis and hypoxia have been documented. EOS produce VEGF and we have recently shown that they are pro-angiogenic cells. However, no study has been performed to verify the existence of a direct link between EOS, hypoxia and angiogenesis in AI.

Objectives: To characterize EOS function and angiogenic potential under hypoxic conditions *in vitro*.

Methods: Human peripheral blood EOS were cultured in normoxic or hypoxic (<3% O2) conditions with or without GM-CSF (20ng/ml). The following parameters were evaluated: Viability (trypan blue and by flow cytometery of annexin V/PI); HIF-1α levels and MAPK phosphorylation (immunoblot); IL-8 and VEGF (ELISA). Nasal polyps were obtained from patients undergoing polypectomy. The polyps were minced, enzymatically digested and cell suspension was subjected to negative selection (anti-CD3/CD14/CD16). Enriched tissue EOS were cultured with or without GM-CSF and in normoxia/hypoxia.

Results: Hypoxia did not influence blood EOS survival but increased HIF-1α levels and VEGF release (normoxia: undetectable; hypoxia: 60.0±17.9 pg/ml; $p<0.05$) particularly in the presence of GM-CSF (normoxia: 73.5±36.2; hypoxia: 186.5±33.9 pg/ml; $p<0.05$). GM-CSF increased MAPK phosphorylation particularly ERK1/2. The ERK1/2 inhibitor PD98059 significantly decreased VEGF release. In the presence of GM-CSF in normoxia, VEGF levels decreased from 42±12.7 to 22.9±9.9 pg/ml, and in hypoxia from 165.6±63.8 to 23.8±6.2 pg/ml. Also with the p38 inhibitor SB203580 decreased VEGF release. PD98059 caused HIF-1α expression decrease both in normoxia and in hypoxia. In hypoxia HIF-1α expression was similar to the one in normoxia. SB20358 slightly decreased HIF-1α expression. To study tissue EOS viability under hypoxic conditions nasal polyps were enzymatically digested and negative selection was performed yielding EOS at 88% viability and at a purity of 66%. Tissue derived EOS were cultured in normoxia or hypoxia for 24h; viability was checked and found to be in medium alone 87% in normoxia and 94% in hypoxia; with GM-CSF 87% in normoxia and 95% in hypoxia.

Conclusions: We have demonstrated that blood and tissue EOS are viable in hypoxia. Blood EOS respond to hypoxia by releasing increased amounts of pro-angiogenic factors and by up-regulating HIF-1α expression. This effect is augmented by the presence of GM-CSF and involves MAPK phosphorylation. These results show the importance of EOS as effector cells of AI characterized by both angiogenesis and hypoxia.

A.H. Nissim Ben Efraim acknowledges a travel grant from the Alex Grass Center for Drug Design and Synthesis of Novel Therapeutics at the Hebrew University. This project was funded by grants from the Aimwell Charitable Trust (UK); The David R. Bloom Center of Pharmacy of HUJI, The David Sydney Davis Fund for Emphysema Related Research and a donation in memory of Mrs. Muriel Turk (UK).

POSTER #12

EOSINOPHIL DEGRANULATION IS INHIBITED BY INTERACTION WITH RESPIRATORY SYNCYTIAL VIRUS

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Background: Respiratory syncytial virus (RSV) is a negative sense, non-segmented RNA virus that can result in severe respiratory infections in normal infants as well as in immunocompromised hosts. Eosinophil recruitment to the lungs has been reported in cases of severe RSV infection, predominantly in young infants (< 3 months of age), and eosinophils can reduce the infectivity of RSV for target cells in tissue culture; likewise, pulmonary eosinophilia was a prominent response among infants undergoing natural RSV infection after immunization with formalin-inactivated RSV vaccine antigens.

Objectives: As part of a larger exploration of the interactions between eosinophils and RSV, we have found that challenge with RSV virions inhibits eosinophil degranulation.

Methods: Human eosinophils (> 98% purity) isolated from heparanized blood were maintained in culture medium with 25 ng/ml of recombinant human interleukin-5 and were challenged for 24 hrs with live RSV or medium alone. Eosinophil granule proteins were detected in culture supernatants by quantitative ELISA.

Results: We determined that challenge with live RSV results in a dose-dependent inhibition of eosinophil degranulation, determined by detection of the granule proteins EDN (eosinophil-derived neurotoxin) and ECP (eosinophil cationic protein). Diminished degranulation is not replication-dependent, as it is also observed in response to UV-inactivated RSV.

Conclusions: Consistent with the findings of Davoine *et al.* (JACI 2008), eosinophils do not degranulate in response to RSV virions alone. In contrast, we observe diminished release of granule proteins EDN and ECP in response to both live and inactivated RSV virions. We are interested in determining the molecular mechanism of this response, specifically which virus proteins have a primary impact on modulating the extent of eosinophil degranulation.

This research is funded by NIAID Division of Intramural Research.

POSTER #13

EOTAXIN-MEDIATED MOUSE EOSINOPHIL DEGRANULATION AND SECRETION OF EOSINOPHIL-ASSOCIATED RNASES

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Background: The secondary granules of human eosinophils contain cytotoxic proteins including MBP, EPO and the EDN and ECP RNases that can be secreted following stimulation as part of host defense and other immune responses. Three processes of granule protein secretion are recognized for human eosinophils: cytolysis, classical exocytosis and piecemeal degranulation (PMD). PMD, in which intact eosinophils completely or partially empty granules and selectively secrete performed mediators by transporting small packets of materials from the cytoplasmic granules to cell surface, is the principal mechanism for human eosinophil degranulation. Unlike extensively studied human eosinophils, both the capacity and mechanisms underlying eosinophil degranulation/secretory responses of murine eosinophils have been poorly investigated and remain controversial. Lack of commercial antibodies specific to mouse eosinophil granule proteins, like the mouse eosinophil-associated RNases (EARs) or MBP, has impeded studies of secretory responses of murine eosinophils. Moreover, studies of moderate degranulation responses of murine eosinophils with physiological stimuli requires a sensitive assay to quantitate secretory responses. Therefore, we applied a new methodology to detect degranulation by measuring secretion of EARs from stimulated murine eosinophils.

Objectives: To investigate the capacity and mechanisms underlying eosinophil degranulation/secretory responses of murine eosinophils following physiological stimulation by cytokines and chemokines.

Methods: Secretory responses of murine eosinophils were assayed using a sensitive novel RNase activity assay (with a fluorescent RNA probe) to detect secreted EARs from stimulated eosinophils, purified from IL-5 transgenic mouse spleens. Secretory responses and mechanisms, validated by assays of secreted eosinophil-peroxidase activity, were evaluated by transmission electron microscopy (TEM).

Results: Murine eosinophils “degranulated” and secreted following stimulation with eotaxin and/or GM-CSF as well as with non-physiological stimulus (phorbol ester), as detected by increases in RNase and EPO activities in cell supernatants. Both methods for assaying secreted products correlated with each other, but RNase assays were more sensitive and thus more suitable for moderate degranulation/secretory responses found with murine eosinophils. Eotaxin-mediated mouse eosinophil degranulation was both $G_{\alpha\iota}$ and CCR3-dependent. TEM revealed signs of degranulation associated with PMD in eotaxin stimulated mouse eosinophils, including significant increase in the number of emptying granules. Other mechanisms of degranulation (granule exocytosis or eosinophil cytolysis) were not detected following eotaxin stimulation.

Conclusions: These data demonstrate that murine eosinophils undergo piecemeal degranulation in response to physiological stimulation with eotaxin and GM-CSF, findings that are pertinent to murine models of eosinophil-associated diseases.

This project was funded by grants from NIH: AI020241

POSTER PRESENTATIONS SESSION II

POSTER #14

NOTCH SIGNALING UNDERLIES FULL AND SUSTAINED EOSINOPHIL ACTIVITIES

Amy L. Radke[1], Lauren L. Reynolds[1], Peter F. Weller[1] and Lisa A. Spencer[1]

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Background: Notch signaling is an evolutionarily conserved pathway critical in directing cell fate determinations throughout development and hematopoiesis. More recently, Notch activation-dependent functions of mature cells have been increasingly appreciated, and Notch signaling is now implicated in diverse physiological processes, including immune modulation, and in multiple disease states, including fibrosis and oncogenesis. We have recently demonstrated that human blood eosinophils express Notch ligands and receptors, suggesting mature eosinophils might maintain a capacity for autocrine Notch signaling.

Objectives: To determine whether autocrine Notch signaling contributes to critical functions of human eosinophils.

Methods: Studies were performed using human blood eosinophils isolated from both atopic and non-atopic donors. To confirm whether human eosinophil-expressed Notch ligands and receptors are functional, complementary approaches involving flow cytometry of permeabilized eosinophils to detect activated intracellular domains of the Notch receptor, and real time RT-PCR to measure induction of Notch-responsive genes were performed. To determine whether autocrine Notch signaling contributes to eosinophil activation, human eosinophils were stimulated with GM-CSF in the presence or absence of Notch signaling inhibitors (three distinct inhibitors utilized) or neutralizing antibodies targeting specific Notch receptors or ligands. Functional outcomes measured included eosinophil viability (as assessed by PI and Annexin V staining), cell shape changes (as assessed morphologically by phase microscopy, flow cytometry and actin staining), and chemokinetic potential (as assessed by measuring migration through transwell filters).

Results: Eosinophil-expressed Notch ligands and receptors are fully functional, and autocrine Notch activation occurred in GM-CSF-stimulated eosinophil cultures. Notch signaling, specifically involving Notch receptor 1, was required for full and sustained GM-CSF-induced cell polarization and chemokinesis, and promoted eosinophil apoptosis.

Conclusions: We have identified Notch signaling as a critical auto-regulatory pathway mediating GM-CSF-induced eosinophil functions.

This project was funded by an Interest Section Award and the Women in Allergy Junior Faculty Development Award to L.A.S. from the American Academy of Asthma, Allergy and Immunology, and by National Institutes of Health grants AI020241 and AI051645 to P.F.W.

POSTERS: SESSION III

The eosinophil: A central player in innate immunity? (Posters 15-19)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
15	**Akuthota, Praveen**	**ANTIGEN PRESENTATION BY HUMAN EOSINOPHILS IS DEPENDENT ON LIPID RAFTS**	**Praveen Akuthota, Lisa A. Spencer and Peter F. Weller**
16	**Andersson, Kirstin**	**FOOD ALLERGENS CONSTITUTE DANGER SIGNALS FOR HUMAN EOSINOPHILS**	**Kerstin Andersson, Elin Redvall, Said Elsayed , Christine Wennerås**
17	**Dyer, Kimberly**	**PNEUMOVIRUS INFECTION OF EOSINOPHILS: MYD88-MEDIATED VIRAL REPLICATION AND CYTOKINE RELEASE**	**Kimberly D. Dyer, Caroline M. Percopo, Stanislaw J. Gabryszewski, and Helene F. Rosenberg**
18	**Kato, Masahiko**	**DIFFERENT CYTOKINE PROFILE AND EOSINOPHIL ACTIVATION ARE INVOLVED IN RHINOVIRUS- AND RESPIRATORY SYNCYTIAL VIRUS-INDUCED ACUTE EXACERBATION OF CHILDHOOD WHEEZING**	**Masahiko Kato, Hiroyuki Tsukagoshi, Masakazu Yoshizumi, Mika Saitoh, Kunihisa Kozawa, Yoshiyuki Yamada, Kenichi Maruyama, Yasuhide Hayashi, and Hirokazu Kimura**
19	**Rigaux, Peter**	**DIFFERENTIAL EXPRESSION OF TLR AND CYTOKINE GENES IN MACROPHAGES AND EPITHELIAL CELLS IN RESPONSE TO PVM INFECTION: COMPARISON TO EOSINOPHILS**	**Peter Rigaux, Zhijun Qiu and Helene Rosenberg**

POSTER #15

ANTIGEN PRESENTATION BY HUMAN EOSINOPHILS IS DEPENDENT ON LIPID RAFTS

Praveen Akuthota [1], Lisa A. Spencer [2] and Peter F. Weller [2]

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Background: Eosinophils have been increasingly recognized to have functions in allergic airways inflammation as professional antigen presenting cells (APCs). In other better characterized APCs, MHC Class II has been demonstrated to be dependent on its localization into lipid rafts.

Objectives: We sought to determine if MHC Class II (HLA-DR) localizes to lipid raft domains of human eosinophils and the functional significance of such localization.

Methods: Human eosinophils were purified from healthy donors and stimulated with 100pM GM-CSF. After antibody cross-linking of HLA-DR followed by cell lysis, lipid rafts were isolated by sucrose density gradient fractionation. Western blot for HLA-DR was then performed on raft and non-raft fractions. Immunofluorescence co-localization studies were performed by incubating eosinophils with fluorescent cholera toxin B (CTB) to label lipid rafts and anti-HLA-DR antibody followed by fluorescent secondary antibody. Lipid raft disruption was achieved by incubating eosinophils with methyl-β-cyclodextrin (MβCD). Flow cytometry and immunofluorescence microscopy were then performed to detect and localize HLA-DR after lipid raft disruption. Functional *in vitro* studies were performed by co-culturing eosinophils loaded with the superantigen staphylococcal enterotoxin A (SEA), either with or without MβCD treatment, and $CD4^+$ T cells from the same donor. T cell stimulation was assessed by CD69 expression at 24 hours and proliferation by CFSE dilution at 96 hours.

Results: We show in purified human eosinophils stimulated with GM-CSF that HLA-DR co-localizes with CTB by immunofluorescence microscopy. Lipid raft disruption with MβCD resulted in a decrease in surface expression of HLA-DR by both flow cytometry and immunofluorescence microscopy. Additionally, HLA-DR was identified by western blot in specific lipid raft fractions isolated by sucrose density gradient fractionation. Finally, we show that lipid raft disruption decreases the ability of eosinophils to stimulate CD4+ T cell activation (CD69 expression) and proliferation (CFSE dilution) through superantigen bound to MHC Class II.

Conclusions: Our results demonstrate that MHC Class II is present in lipid rafts of human eosinophils and that its association with lipid rafts is necessary for optimal APC function.

This project was funded by grants from the National Institutes of Health (NIH AI051645 and NIH AI020241 to Dr. P.F. Weller and T32 training grant NIH HL007633).

POSTER #16

FOOD ALLERGENS CONSTITUTE DANGER SIGNALS FOR HUMAN EOSINOPHILS

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Background: An epidemic increase in the incidence of sensitization to common food stuffs is seen in the Western world. The eosinophilic granulocyte is a key cellular component of allergic reactions, including those directed against foods. It has previously been shown by us and others that eosinophils have inherent reactivity to airborne allergens.

Objectives: To investigate the innate reactivity of eosinophilic granulocytes to common allergenic food stuffs.

Methods: Eosinophils derived from healthy, non-allergic individuals were incubated with food extracts and fractions thereof. Eosinophilic activation parameters such as chemotaxis, degranulation, release of eicosanoids and intracellular calcium fluxes were monitored.

Results: Freshly isolated blood eosinophils were screened for reactivity against the food extracts used to diagnose food hypersensitivity in Sweden. Eosinophils derived from healthy, non-allergic individuals became activated by exposure to cow's milk and cod fish. Whereas milk evoked release of the granule protein eosinophil peroxidase (EPO), cod fish elicited eosinophil migration *in vitro* as well as intracellular calcium mobilization. Both food stuffs triggered the release of prostaglandin E_2 and Leukotriene C_4 from eosinophils. Whey proteins appeared to be the milk fraction recognized by eosinophils. In contrast, the major fish allergen, parvalbumin, was not responsible for eosinophilic activation.

Conclusions: Human blood derived-eosinophils from non food-allergic individuals can recognize and become activated by exposure to food extracts. Distinct eosinophilic activation patterns were triggered by the different food stuffs.

This project was funded by grants from the Swedish Research Council, Cancer and Allergy Foundation, ALF Funding, the Swedish Allergy and Asthma Foundation, Västra Götaland Region Research Funding and Th C Bergh Foundation for Scientific Research.

POSTER #17

PNEUMOVIRUS INFECTION OF EOSINOPHILS: MYD88-MEDIATED VIRAL REPLICATION AND CYTOKINE RELEASE

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Background: Eosinophils are recruited to the lung in response to infection with pneumovirus pathogens and have been associated with both the pathophysiologic sequelae of infection and, more recently, with accelerated virus clearance.

Objectives: We hypothesize that eosinophils can be infected with pneumoviruses and may respond to viral replication by releasing proinflammatory mediators.

Methods: Bone marrow eosinophils were exposed to Pneumonia Virus of Mice (PVM) at an MOI=1-10 for 2 hours at 37°C and then related in fresh media containing 10 ng/mL rmIL-5. At the time points indicated, aliquots of culture media were collected for analysis of cytokine release. Aliquots of cells were collected for RNA isolation for determination of virus titer and for total protein isolation for Western blots.

Human eosinophils were isolated from whole blood using the Miltenyi human eosinophil isolation kit. One million eosinophils maintained in 25 ng/mL recombinant human IL-5 were treated with varying inocula of RSV-A or heat-inactivated virus for 24 hours at 37°C. The supernatants were collected for analysis of cytokine release and eosinophils were evaluated for virus titer. Stocks of recombinant green RSV (rgRSV^{27}, passage 4) were amplified in HEp-2 cells and used to infect isolated human eosinophils at an MOI < 2 pfu per cell. Green fluorescent cells were detected after 4 days and photographed at 7 days.

Results: Pneumonia virus of mice (PVM) replicates in mouse eosinophils. Replication within eosinophils elicits release of disease-related proinflammatory mediators including IL-6 and IP-10. PVM replication is accelerated 10-fold in MyD88 gene-deleted eosinophils in association with diminished release of proinflammatory cytokines IL-6, IP-10, CCL3, and CCL2. PVM infection has no impact on cell viability, percentage eosinophils, or cytokine gene transcription over the time course studied. Likewise Respiratory Syncytial Virus (RSV) appears to replicate in human peripheral eosinophils. Green fluorescent protein can be detected in human eosinophils challenged with rgRSV and RSV elicits the release of IL-6 from eosinophils infected with actively-replicating virus.

Conclusions: We have shown that eosinophils are targets of virus infection and thus they may contribute in varied and complex ways to the pathogenesis and resolution of pneumovirus disease *in vivo*.

POSTER #18

DIFFERENT CYTOKINE PROFILE AND EOSINOPHIL ACTIVATION ARE INVOLVED IN RHINOVIRUS- AND RESPIRATORY SYNCYTIAL VIRUS-INDUCED ACUTE EXACERBATION OF CHILDHOOD WHEEZING

Masahiko Kato[1], Hiroyuki Tsukagoshi[4], Masakazu Yoshizumi[4], Mika Saitoh[4], Kunihisa Kozawa[4], Yoshiyuki Yamada[1], Kenichi Maruyama[2], Yasuhide Hayashi[3], and Hirokazu Kimura[5]

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Background: Little information is available on eosinophil activation and cytokine response in virus-induced wheezing/asthma.

Objectives: We attempted to detect respiratory viruses and measure eosinophil cationic protein (ECP), and 27 types of cytokines/chemokines in both serum and nasal secretions from children with wheezing.

Methods: We detected viruses in nasal secretions from 174 patients with acute exacerbations of wheezing using antigen detection kits or reverse transcription-polymerase chain reaction, followed by direct DNA sequencing analysis. We measured peripheral eosinophil counts, and serum concentrations of ECP and 27 cytokines /chemokines (IL-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN-γ, IP-10, TNF-α, GM-CSF, G-CSF, MCP-1, MIP-1α, MIP-1β, eotaxin, RANTES, PDGF-bb, FGF basic, and VEGF) using a multiplex beads-based assay in patients with or without wheezing. We also examined nasal ECP and 27 cytokines/chemokines in wheezing patients.

Results: Of 174 samples from wheezing exacerbations, rhinovirus was detected in 59; respiratory syncytial (RS) virus in 44; enterovirus in 17; other viruses in 19; and no viruses in 35. Serum concentrations of ECP, IL-5, IL-6, IL-8, IL-1rα, and IP-10 were significantly elevated in rhinovirus-induced wheezing compared with without wheezing. Similarly, serum ECP, IL-5, and IP-10 were significantly higher in rhinovirus-induced wheezing than in controls. On the other hand, IL-1rα and IP-10, but not ECP and IL-5 were significantly higher in RS virus-induced wheezing than in controls. Furthermore, only IL-5 was significantly elevated in the rhinovirus group compared with the RS virus groups in both serum and nasal secretions.

Conclusions: Virus-induced wheezing/asthma, especially via rhinovirus, might enhance eosinophil activation through IL-5 production.

This project was funded by a grant from the Grants-in-Aid for Scientific Research (C) (#18591208, #20591267) from the Ministry of Education, Culture, Sports, Sciences and Technology; the Ministry of Environment Government of Japan; and Gunma Prefecture, Japan.

POSTER #19

DIFFERENTIAL EXPRESSION OF TLR AND CYTOKINE GENES IN MACROPHAGES AND EPITHELIAL CELLS IN RESPONSE TO PVM INFECTION: COMPARISON TO EOSINOPHILS

Peter Rigaux[1*], Zhijun Qiu[1*] and Helene Rosenberg[1]

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Background: Respiratory Syncytial Virus (RSV) is one of the leading causes of respiratory infection in children. Our laboratory has previously reported that Pneumonia Virus of Mice (PVM), a mouse pathogen of the same virus family as RSV, can replicate in cultured mouse eosinophils (Dyer *et al., J. Immunol*., 2008). Interestingly, PVM replication is accelerated in eosinophils that are of the TLR common signaling adapter, MyD88 (Dyer *et al., in review*, 2009).

Objectives: Because lung epithelial cells and pulmonary macrophages are recognized as primary targets of PVM infection *in vivo*, our aim is to evaluate PVM replication and gene expression in the RAW 264.7 macrophage and MLE-12 epithelial cell lines and to evaluate differential expression of cytokines and TLR signaling related genes during PVM infection. Moreover, we aimed to determine the requirement of MyD88 in this process.

Methods: MyD88 expression in RAW 264.7 and MLE-12 cells was knocked down using puromycin-resistant Mission lentivirus shRNA system (Sigma). Cell lines were then challenged with PVM (actively-replicating, heat-inactivated virus, or no virus). RNA was isolated at different time points to monitor PVM replication by qRT-PCR. Gene expression was assessed by RT-PCR array 7 days after challenge.

Results: Maximum virus titer was observed at 7 days post-challenge in both RAW 264.7 and MLE-12 cell lines. PVM replication in RAW 264.7 macrophages resulted in augmented expression of transcripts encoding genes associated with TLR signaling (TLR2, TLR3, TLR6, Btk, Nfκb1, Nfκb2) and Fadd, which has a role in apoptosis and in viral recognition through the RIG-I/MDA5 signaling pathway. PVM replication likewise results in augmented expression cytokines and chemokines, including CCl2, CXCL10, CSF2, IL-6RA; interestingly, CCL2 (MCP-1) and CXCL10 (IP-10) are also released by eosinophils in response to PVM infection. In contrast to what was observed with MyD88 -/- eosinophils, suppression of MyD88 in the RAW 264.7 macrophage cell line resulted in diminished PVM replication, and initial results suggest that suppression of MyD88 expression had no substantial impact on gene expression.

Conclusions: While PVM infection in RAW 264.7 macrophages shares features in common with eosinophils, including replication-dependent release of cytokines CCL2 and CXCL10, dependence on TLR / MyD88 signaling is substantially different. As such, it would be interesting to characterize the role of the alternate RIG-I/MDA5 pathway during PVM replication.

This project was supported by funding from NIAID Division of Intramural Research
*These authors contributed equally to this work.

POSTERS: SESSION V

Eosinophil / T cell interactions in health and disease (Poster 20)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
20	**Prussin, Calman**	**DIFFERENTIAL TH2 RESPONSES ALTERNATIVELY DRIVE EOSINOPHILIC VS. ANAPHYLACTIC FOOD ALLERGY**	**Calman Prussin, Joohee Lee and Barbara Foster**

POSTER #20

DIFFERENTIAL TH2 RESPONSES ALTERNATIVELY DRIVE EOSINOPHILIC VS. ANAPHYLACTIC FOOD ALLERGY

Calman Prussin[1], Joohee Lee[1] and Barbara Foster[1]

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Background: Both eosinophil associated gastrointestinal disorders (EGIDs) and anaphylactic food allergy are associated with Th2 and IgE responses, yet are clinically distinct in being manifested by gut eosinophilia and anaphylaxis, respectively.

Objectives: To determine if the clinical differences between anaphylactic food allergy and EGIDs are reflected in different Th2 responses to foods.

Methods: Peanut allergic (PA), allergic eosinophilic gastroenteritis (AEG), nonatopic (NA), and non-atopic EG (NA-EG) subjects were enrolled. Antigen specific CD4 T cell responses to peanut, soy, shrimp and staphylococcal enterotoxin B were measured using intracellular cytokine staining and polychromatic flow cytometry with simultaneous measurement of IL-4, IL-5, IL-13, IFN-γ and TNF.

Results: Although 7 combinations of Th2 cytokines are theoretically possible, only two distinct subpopulations of Th2 cells were found: IL-5^+ Th2 (IL-5^+, IL-4^+, IL-13^+) and IL-5^- Th2 (IL-5^-, IL-4^+, IL-13^+) cells. Peanut specific IL-5^+ Th2 cells were present at a 20-fold greater frequency in AEG vs. PA (0.0081% vs. 0.00039%, p=0.05), whereas similar frequencies of IL-5^-, Th2 cells were found (0.0067% vs. 0.0041%). For all food Ags, significantly greater frequencies of IL-5^+ Th2 cells were found in AEG than in PA subjects. Similar very low magnitude Th1 responses to all foods were common to all subject groups. In AEG vs. PA, IL-5^+ Th2 responses accounted for 45% vs. 9% respectively, of the total food allergen specific Th2 response. In PA but not AEG, IL-5^- Th2 responses to peanut were highly correlated with peanut specific IgE (r= 0.87 vs. 0.55, respectively).

Conclusions: Th2 responses are composed of two subpopulations: IL-5^+ Th2 and IL-5^- Th2 cells. IL-5^+ Th2 food allergen specific T cell responses are singularly associated with AEG, whereas PA is associated with a dominant IL-5^- Th2 response. These results suggest heterogeneity within Th2 responses that may alternatively favor IgE production versus eosinophilic inflammation (e.g. pro-anaphylactic vs. pro-eosinophilic T cells). Different factors may alternatively favor the generation of one or the other Th2 subpopulation, leading to clinically diverse allergic disease.

This project was funded by the National Institute of Allergy and Infectious Diseases, NIH, Intramural Research Program.

POSTERS: SESSION VI

Novel findings on cytokines, chemokines, and other mediators involved in eosinophil biology and eosinophil-associated disorders (Posters 21-26)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
21	**Buels, Kalmia**	**PARASYMPATHETIC NERVES EXPRESS CCR3 CHEMOKINES THAT ARE POTENTIATED BY TNF α**	**Kalmia S. Buels, David B. Jacoby and Allison D. Fryer**
22	**Flamand, Nicolas and Laviolette, Michel**	**DIFFERENTIAL EFFECTS OF EOTAXINS ON THE TRANSMIGRATION OF HUMAN EOSINOPHILS**	**Véronique Provost, Anick Langlois, Marek Rola-Pleszczynski, Nicolas Flamand, Michel Laviolette**
23	**Flamand, Nicolas**	**THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL ACTIVATES HUMAN EOSINOPHILS THROUGH SEVERAL MECHANISMS: IMPORTANCE OF EICOSANOIDS.**	**Flamand N, Ferland C, Dupuis L, Chouinard F, Langlois A, and Laviolette M.**
24	**Redvall, Elin**	**INTERPLAY BETWEEN SIGNALING VIA THE FORMYL PEPTIDE RECEPTOR (FPR) AND CHEMOKINE RECEPTOR 3 (CCR3) IN HUMAN EOSINOPHILS**	**Elin Redvall, Lena Svensson, Marianne Johnsson, Anna-Lena Stenfeldt, Claes Dahlgren and Christine Wennerås**
25	**Ueki, Shigeharu**	**LEPTIN HAS PRIMING EFFECT ON EOTAXIN-INDUCED EOSINOPHIL CHEMOTAXIS**	**Shigeharu Ueki, Hikari Kato, Junko Kihara, Masahide Takeda, Tomomi Tanigai, Wataru Ito, Hiroyuki Kayaba and Junichi Chihara**
26	**Zhu, Yiming**	**THE HUMAN PROMYELOBLASTIC CELL LINE HL-60 CLONE 15 CAN SERVE AS A MODEL SYSTEM FOR THE STUDY OF CYTOKINE PRIMING OF CHEMOATTRACTANT ACTIONS IN HUMAN BLOOD EOSINOPHILS**	**Yiming Zhu, Paul J. Bertics**

POSTER #21

PARASYMPATHETIC NERVES EXPRESS CCR3 CHEMOKINES THAT ARE POTENTIALTED BY TNF α

Kalmia S. Buels[1], David B. Jacoby[1, 2] and Allison D. Fryer[1, 2]

[1]Physiology and Pharmacology, [2]Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR, USA.

Background: Eosinophils cluster around parasympathetic nerves in asthmatic lungs and in antigen challenged guinea pigs where they mediate airway hyperreactivity. Blocking CCR3 receptors in antigen challenged guinea pigs prevents airway hyperreactivity and decreases the number of eosinophils associated with nerves in the lungs. Airway nerves are known to express CCL11 (Fryer et al., 2006 JCI 116:228) and CCL26 (Chou et al., 2005 AJRCMB 33:1). However, it is not known whether they make other chemokines known to interact with CCR3.

Objectives: To determine whether known CCR3 chemokines including CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL24, CCL26, and CCL28 are expressed in airway parasympathetic nerves or induced by tumor necrosis factor alpha (TNFα), a cytokine increased in asthma.

Methods: Human neuroblastoma cells (SK-N-SH) and primary cultures of tracheal parasympathetic nerves, isolated from either sensitized (to ovalbumin) or non-sensitized guinea pigs, were treated with species-specific recombinant TNFα [human (3 to 3000 U/mL); guinea pig (0.05 to 50 ng/mL)] for 0-48 h. mRNA was isolated from SK-N-SH cells, or from cell bodies for parasympathetic nerves, and chemokine expression was measured using real-time PCR. In addition, chemokines released in the SK-N-SH cell culture supernatants were measured by ELISA.

Results: We found that SK-N-SH cells express mRNA for CCL5, CCL7, CCL8, and CCL11. Furthermore, TNFα increased mRNA expression for these same chemokines in a time and concentration dependent manner. Protein expression for three of these four chemokines (CCL5, CCL7, and CCL11) was also increased. Guinea pig parasympathetic neurons expressed CCL5, CCL7, and CCL11. TNFα increased CCL7 mRNA in a concentration dependent manner. There was a greater TNFα induced increase in CCL7 mRNA expression in parasympathetic nerves isolated from sensitized guinea pigs compared to non-sensitized guinea pigs.

Conclusions: Nerves, including parasympathetic nerves, express a subset of CCR3 chemokines that can be increased by TNFα depending on sensitization status. These data suggest a mechanism whereby neurons can actively recruit eosinophils and subsequently mediate neuronal hyperreactivity in asthma that may be altered by atopic status.

This project was funded by a grant from the Tartar Trust (KSB) and NIH grants HL55543, ES01460, HL54659, HL071795.

POSTER #22

DIFFERENTIAL EFFECTS OF EOTAXINS ON THE TRANSMIGRATION OF HUMAN EOSINOPHILS

Véronique Provost[1], Anick Langlois[1], Marek Rola-Pleszczynski[2], Nicolas Flamand[1], Michel Laviolette[1],

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Background: The bronchial inflammation observed in the mucosa of asthmatics is characterized by an eosinophilic infiltration. Among the best chemoattractant of eosinophils are the chemokines eotaxin-1, -2, and -3. Eotaxin-2 and -3 were recently described while eotaxin-1 has been known for more than a decade. Although they can activate the same receptor (CCR3), the differential effect of eotaxins on eosinophil transmigration has not been deeply characterized yet.

Objective: To compare the impact of the different eotaxins on the activation of human eosinophils.

Methods: Eosinophils were isolated from the peripheral blood of healthy or asthmatic volunteers. Transmigration assays through cell culture inserts coated with (or without) a reconstituted basement membrane (Matrigel^{TM}) were induced by eotaxin-1, -2 or -3. Dose response and kinetic experiments of eosinophil activation were also performed.

Results: All eotaxins induced eosinophil transmigration and their effect on chemotaxis was similar. Interestingly, eotaxins induced the transmigration of eosinophils through Matrigel^{TM} with a different efficiency (eotaxin-3 > eotain-1 > eotaxin-2) and potency (eotaxin-1 ≅ eotaxin-2 > eotaxin-3). Kinetic experiments show that the eotaxin-1-induced transmigration of eosinophils was delayed compared to eotaxin-2 and eotaxin-3. Interestingly, the effect of eotaxin-1 and -3 were more pronounced in asthmatic eosinophils compared to eosinophil from healthy subjects.

Conclusions: These results show that even if eotaxins likely activate eosinophils through the same cell surface receptor (CCR3), these chemokines have distinct effects on their transmigration. We are now investigating the underlying responsible for these discrepancies.

POSTER #23

THE ENDOCANNABINOID 2-ARACHIDONOYL-GLYCEROL ACTIVATES HUMAN EOSINOPHILS THROUGH SEVERAL MECHANISMS: IMPORTANCE OF EICOSANOIDS.

Flamand N, Ferland C, Dupuis L, Chouinard F, Langlois A, and Laviolette M.

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Background: Endocannabinoids such as 2-arachidonoyl-glycerol (2-AG) are lipid mediators with documented effects in the regulation of pain and neurological disorders. Moreover, they show an intriguing profile of anti- and pro-inflammatory effect. This might be related to the arachidonoyl moiety contained in their structure, arachidonic acid being the precursor of several pro- and anti-inflammatory eicosanoids such as the leukotrienes (LTs) and the prostaglandins. Since endocannabinoids are hydrolyzed rapidly into arachidonic acid, we hypothesized that their hydrolysis would lead to eicosanoid biosynthesis and that the eicosanoids generated *de novo* would modulate the immune response of activated leukocytes.

Objectives: This study has two main objectives: 1) to evaluate the impact of endocannabinoids on lipid mediator biosynthesis; 2) to determine whether eicosanoids are implicated in the modulation of eosinophil responses by endocannabinoids.

Methods: Human eosinophils were isolated from the peripheral blood of asthmatics volunteers and were incubated with endocannabinoids. The biosynthesis of lipid mediators was analyzed by EIA. Immunoblot and RT-PCR experiments were also performed. Boyden Chambers were utilized when chemotaxis experiments were performed,

Results: 2-AG induced the biosynthesis of LTC_4 and 14,15-LTC_4 as potently and efficiently than arachidonic acid. The observed biosyntheses were inhibited by endocannabinoid biosynthesis inhibitors. 2-AG also induced the chemotaxis of eosinophils and this chemotaxis was blocked by cannabinoid receptor antagonists, endocannabinoid hydrolysis inhibitors, the 5- and 15-lipoxygenase inhibitor NDGA but not by the 5-lipoxygenase inhibitor L-739,010. Importantly, cannabinoid receptor agonists modestly induced the chemotaxis of eosinophils, suggesting that the 2-AG-induced chemotaxis of human eosinophils was the consequence of a mixed effect involving cannabinoid receptors, 2-AG hydrolysis, and 15-lipoxygenase. 2-AG and cannabinoid receptor agonists also induced the activation of the ERK pathway.

Conclusions: Our data demonstrate that 2-AG activates human eosinophils *ex vivo*. This activation implicates its hydrolysis into arachidonic acid (eicosanoid biosynthesis and chemotaxis), and the activation of cannabinoid receptors (chemotaxis, ERK). The data support a pro-inflammatory role of 2-AG in the regulation of eosinophil functions.

POSTER #24

INTERPLAY BETWEEN SIGNALING VIA THE FORMYL PEPTIDE RECEPTOR (FPR) AND CHEMOKINE RECEPTOR 3 (CCR3) IN HUMAN EOSINOPHILS

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Background: We have shown that eosinophils express two members of the formyl peptide receptor family, the formyl peptide receptor (FPR) and formyl peptide receptor like-1. Both which bind the tripeptide fMLF. CCR3 is a key chemokine receptor in eosinophils, which binds to eotaxin-1.

Objectives: To identify the signaling pathways used by CCR3 and FPR and investigate whether there is interplay between these two receptors in human eosinophils.

Methods: Human eosinophils were purified from heparinized blood from healthy volunteers. The impact of blockade and desensitization of the two receptors on effector functions such as chemotaxis and respiratory burst was investigated. Flow cytometry-based methods were used to calculate receptor density and ligand binding to the respective receptors. In order to see whether CCR3 and FPR co-localized, confocal microscopy and fluorescence resonance energy transfer were used.

Results: Signaling through the FPR caused unidirectional downregulation of CCR3-mediated chemotaxis, but not respiratory burst. This was not caused either by reduced ability of eotaxin-1 to bind to CCR3, nor by downregulation of CCR3 from the cell surface. Confocal microscopy and adFRET analysis ruled out homo- or heterodimer formation between FPR and/or CCR3 as an explanation for the reduction in chemotaxis via CCR3. Pharmacologic inhibition of signal transduction molecules showed that the release of free oxygen radicals in response to eotaxin-1, compared to fMLF, was relatively more dependent on the p38 MAPK pathway.

Conclusions: fMLF is an end-point chemoattractant in eosinophils and thus there is unidirectional cross-talk between the FPR and CCR3. Further investigation is needed to elucidate the signaling pathways used by those two receptors and how and where they diverge to signal different eosinophilic responses.

This project was funded by the Swedish Research Council (K2005–06X-14180–04A), The Swedish Cancer and Allergy Foundation, LUA-SAM (7158), The Swedish Asthma and Allergy Association's Research Foundation,Wilhelm and Martina Lundgren's Foundation, Consul Th. C. Bergh's Foundation for Scientific Research, The Foundation for Scientific Research in Memory of Karl and Annie Leon and The Foundation Blanceflor Boncompagni-Ludovisi.

POSTER #25

LEPTIN HAS PRIMING EFFECT ON EOTAXIN-INDUCED EOSINOPHIL CHEMOTAXIS

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Background: To date, a number of epidemiological studies have shown the association between asthma and obesity. Leptin is mainly produced by white adipose tissue and serum leptin markedly increased in obese humans correlating to BMI. Leptin is also known to act in the hypothalamus to induce satiety and increase metabolism. Recently, cross-sectional studies have suggested the association of leptin and asthma, showing higher serum leptin in patients with asthma independently of obesity.

Objectives: In this study, we examined whether the exposure to leptin might affect the eosinophil chemotactic response.
Methods: We first investigated the leptin-directed eosinophil migration by using Boyden chambers. Next, eosinophils were exposed to leptin in a wide range of concentrations for 1 h, and then eotaxin-induced chemotaxis was examined. Western blotting for the detection of phosphorylation of ERK1/2 and p38 was performed to determine whether enhanced activation of signaling pathway resulted in enhanced eotaxin-directed chemotaxis by leptin. Calcium flux was also assessed using luminometor.

Results: Leptin dose-dependently induced eosinophil chemotaxis. Eotaxin effectively induced eosinophil chemotaxis, and we found that the physiological concentrations of leptin amplified the chemotactic response to eotaxin. Although leptin did not affect the eotaxin-induced phosphorylation of ERK1/2 and p38, the cells incubated with leptin had a greater calcium mobilization compared with the medium control by stimulation with eotaxin.

Conclusions: These results suggest the proinflammatory roles of leptin on activation process of eosinophil by locally produced chemokine. Our study adds additional information on the molecular mechanisms of association between asthma and obesity.

This study was funded in part by Grants-in-Aid for Scientific Research and a grant from "The 21st Century Center of Excellence (COE) Program" supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

POSTER #26

THE HUMAN PROMYELOBLASTIC CELL LINE HL-60 CLONE 15 CAN SERVE AS A MODEL SYSTEM FOR THE STUDY OF CYTOKINE PRIMING OF CHEMOATTRACTANT ACTIONS IN HUMAN BLOOD EOSINOPHILS

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Background: Exposure of human peripheral blood eosinophils to IL-5 family cytokines (IL-5, IL-3, GM-CSF) is well-recognized to prime/potentiate eosinophil responsiveness and ERK1/2 activation following stimulation with chemoattractants. However, defining the intracellular mechanisms underlying this process has been impeded by the limitations associated with the molecular manipulation of human primary eosinophils. Thus, to establish a more tractable cell model for mechanistic examination of human blood eosinophil signaling, we evaluated a human promyeloblastic cell line, HL-60 Clone 15 (HC), which has been reported to differentiate into eosinophil-like cells that express eosinophilic granule proteins and eosinophil-specific cytokine receptors.

Objectives: The current study is to characterize IL-5 family cytokine- and chemoattractant-induced ERK1/2 activation in HC and to evaluate whether HC could serve as an *in vitro* model for studying the mechanisms of priming in human blood eosinophils.

Methods: Native or differentiated (sodium butyrate, 5 days) HC were stimulated with IL-5 family cytokines, fMet-Leu-Phe (fMLP) and/or CCL5 for various time periods. The cells were then lysed and evaluated for ERK1/2 phosphorylation using immunoblotting and densitometric analysis. In experiments assessing priming, cells were pretreated with IL-5 family cytokines followed by stimulation with fMLP. Purified human blood eosinophils were examined in parallel experiments. To ascertain the surface expression of cytokine receptors, purified human blood eosinophils, native and differentiated HC were preincubated with PE-conjugated antibodies or IgG controls, followed by analysis using flow cytometry.

Results: Native HC exhibited minimal responses to IL-5, fMLP or CCL5, but did respond to IL-3 treatment alone. In contrast, ERK1/2 were phosphorylated in differentiated HC cells stimulated with IL-5 (15 min and 30 min, N=6, $p<0.04$), fMLP (2 min and 5 min, N=4) or CCL5 (2 min and 5 min, N=3). Furthermore, the kinetics of ERK1/2 phosphorylation in differentiated HC was similar to that in human blood eosinophils. Using flow cytometry, IL-3 receptor α chain was detected at similar levels on the surface of both native and differentiated HC; however, the IL-5 receptor α chain appeared only marginally detectable by flow cytometry. Consequently, IL-3 was more potent at inducing a priming response, namely the synergistic increase of fMLP-stimulated ERK1/2 phosphorylation in both differentiated (N=5, $p<0.02$) and native HC (N=6, $p<0.003$).

Conclusions: These studies are the first to report a cell model system that can recapitulate IL-5 family cytokine-mediated priming of chemoattractant-induced responses (potentiated ERK1/2 phosphorylation) associated with primary human eosinophils. This system should allow for an enhanced capacity to molecularly manipulate the signaling molecules that may be essential for priming, and thereby permit a more rigorous establishment of the mechanisms associated with this key eosinophil response to chemoattractants.

POSTERS: SESSION VII

Eosinophils and Disease (I): Allergic Inflammation (Posters 27-32)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
27	**Collins, Margaret**	**EOSINOPHILIC ESOPHAGITIS (EE) WITH CELIAC DISEASE (CD)**	**Margaret H. Collins, M.D., Guangu Luo, M.D., and Marc E. Rothenberg, M.D., Ph.D.**
28	**DeSouza, Ivani**	**PRE-EXPOSURE TO STAPHYLOCOCCAL ENTEROTOXIN A EXACERBATES THE PULMONARY ALLERGIC EOSINOPHIL RECRUITMENT IN RATS**	**Ivani A. DeSouza Nadia S. Mariano, Glaucia C. de Mello, Tatiane Ferreira, André Schenka, Enilton A. Camargo, and Edson Antunes**
29	**Garcia, Rodolpho**	**THE HUMAN EOSINOPHIL PROTEOME. CHANGES INDUCED BY BIRCH POLLEN ALLERGY**	**Charlotte Woschnagg, Jens Forsberg, Åke Engström, Federico Odreman, Per Venge and Rodolfo C. Garcia**
30	**Lorton, Jesse**	**SENSITIZATION INHIBITS SELECTIVE RECRUITMENT OF EOSINOPHILS TO AIRWAYS AFTER OZONE**	**Jesse K. Lorton, David B. Jacoby and Allison D. Fryer**
31	**Nie, Zhenying**	**OZONE CHANGES AIRWAY EOSINOPHILS**	**Zhenying Nie, Jesse K. Lorton, David, B. Jacoby and Allison, D. Fryer**
32	**Pineton de Chambrun, Guillaume**	**NATURAL HISTORY OF EOSINOPHILIC GASTROENTERITIS**	**Guillaume Pineton de Chambrun, Florent Gonzalez, Jean-Yves Canva, Samia Gonzalez, Lucie Houssin, Antoine Cortot and Jean-Frédéric Colombel**

POSTER #27

EOSINOPHILIC ESOPHAGITIS (EE) WITH CELIAC DISEASE (CD)

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Background: EE is a disorder related to food allergy. CD is a T-cell mediated immune disease caused by an oral antigen, gliadin. Both diseases are reversible following a food-elimination diet. We report several patients who have EE with CD and compare them to patients who have CD or EE only.

Objectives: Identify clinicopathologic characteristics at diagnosis of EE and CD patients (Group 1) compared to CD patients (Group 2), and EE patients (Group 3).

Methods: Patients were identified in clinical and pathology databases. Slides were reviewed to confirm the diagnoses, and to perform quantitative histomorphometry. Lymphocytes were quantitated as number per 100 enterocytes (intraepithelial lymphocytes, IEL) in duodenum. Eosinophils were quantitated as peak eosinophil number (PEN) per high power field (hpf) in all biopsies. Values for groups are expressed as mean±standard deviation. Student t test was performed and $P \leq 0.05$ considered significant.

Results: There were 5 patients (4M, 1F) in Group 1 with mean age 7 years (age range 4-14 years), 12 patients (3M, 9F) in Group 2, mean age 7 years (range 2-14 years), and 8 patients (6M, 2F) in Group 3, mean age 6 years (range 1-16 years). All patients who had CD had Marsh type 3a duodenal histology (i.e, markedly blunt villi with elongated crypts), and increased (≥40) IEL. IEL in duodenal biopsies were not significantly different in Group 1 (69±32) compared to Group 2 (74±40) ($P \geq 0.05$); IEL in Group 3 (10±3) were significantly less than in other groups ($P \leq 0.05$). Following gluten-free diet, IEL were normal in 3/3 Group 1 patients (8±3) and 3/3 Group 2 patients (24±10), duodenal morphology was improved, and all patients were clinically improved. PEN was significantly increased in Group 3 esophageal biopsies (151±64) compared to Group 1 (66±26) ($P \leq 0.05$); eosinophils were not found in Group 2 esophageal biopsies. PEN in Group 1 esophageal biopsies was more variable but remained elevated (89±88) following gluten-free diet and was not different from pre-diet value. CD patients with EE (62±14) or without EE (53±23) had increased PEN in duodenal biopsies compared to patients with EE only (17±12) ($P \leq 0.05$). In 3/3 Group I patients, duodenal PEN was significantly reduced (41±12, $P \leq 0.05$) after gluten-free diet. Gastric PEN was not increased in any group compared to published normal values for pediatric patients.

Conclusions: Duodenal histology of EE with CD is similar to CD only. The immune response to gluten in CD with or without EE includes eosinophilic inflammation in duodenal mucosa that is reduced after gluten is removed from the diet. However, esophageal eosinophilic inflammation persists after gluten-free diet in patients who have both diseases, and EE in those patients should be treated with EE-specific therapy.

POSTER #28

PRE-EXPOSURE TO STAPHYLOCOCCAL ENTEROTOXIN A EXACERBATES THE PULMONARY ALLERGIC EOSINOPHIL RECRUITMENT IN RATS

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[1]Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas (São Paulo), Brazil1; [2] Department of Biology and Physiology, Faculty of Medicine of Jundiaí (FMJ), Jundiaí (São Paulo), Brazil.

Background: Gram-positive *Staphylococcus aureus* releases classical enterotoxins which aggravates allergic airway diseases. However, little is known about the mechanisms underlying the cell influx exacerbation in asthmatic individuals under exposure to Staphylococcal enterotoxins.

Objectives: We therefore aimed to investigate the effects of airways exposure to Staphylococcal enterotoxin A (SEA) to pulmonary leukocyte recruitment in rats sensitized and challenged with ovalbumin (OVA).

Methods: Rats were exposed to SEA at 4 h prior to OVA challenge or at 4 h post-OVA challenge. Leukocyte counts were assayed in bronchoalveolar lavage (BAL) fluid, bone marrow and lung tissue obtained at 24 h after OVA challenge.

Results: Pre-exposure to SEA markedly enhanced the eosinophil counts in both BAL fluid and pulmonary tissue in OVA-challenged rats, whereas neutrophil and mononuclear cell counts remained unchanged. In bone marrow, pre-exposure to SEA alone significantly increased the number of eosinophils, and that was further increased in OVA-challenged rats. Exposure to SEA post-OVA challenge did not affect the number of eosinophils, neutrophils and mononuclear cells in BAL fluid. Pre-exposure to the endotoxin lipopolyssacharide (LPS) in OVA-challenged animals rather enhanced the neutrophil number in BAL fluid. In rats pre-exposed to SEA and OVA-challenged, a marked elevation in the levels of TNF-α (PBS/OVA: 6.4 ± 0.7 and SEA/OVA: 21.3 ± 3.8 pg/mg protein) and eotaxin (PBS/OVA: 0.7 ± 0.3 and SEA/OVA: 69.1 ± 6.7 pg/mg protein). No alterations in the levels of IL-10 in BAL fluid were observed. The eotaxin levels increased by about of 3-fold in alveolar macrophages treated with SEA *in vitro* (501.2 ± 8.8 and 1484.4 ± 38.2 pg/mg protein for untreated and SEA-treated macrophages).

Conclusions: In conclusion, airways pre-exposure to SEA causes a selective increase in eosinophil number in BAL fluid and bone marrow of OVA-challenged rats by mechanisms involving enhancement of TNF-α and eotaxin synthesis.

This Project was funded by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil.

POSTER #29

THE HUMAN EOSINOPHIL PROTEOME. CHENGES INDUCED BY BIRCH POLLEN ALLERGY 7

Charlotte Woschnagg[1], Jens Forsberg[2], Åke Engström[2], Federico Odreman[3], Per Venge[3] and Rodolfo C. Garcia[1,3]

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Background: During pollen allergy and/or asthma, peripheral blood eosinophils show increases in eosinophil migration, the ability to secrete granule proteins and the expression of CD9 and CD11b; priming of adhesion to ICAM-1/VCAM-1; and alterations in oxidative metabolism. These changes are most likely to be reflected by the cell's protein composition. Neither the eosinophil proteome nor changes induced by pollen allergy have been reported.

Objectives: To study the eosinophil proteome and the changes induced by birch pollen allergy at the peak of a season.

Methods: Eosinophils were purified and the proteins contained in cytoplasmic extracts and subcellular fractions were separated bi-dimensionally, characterized in terms of Mr and pI and identified by mass spectrometry. Protein maps of asymptomatic and allergic subjects were compared and proteins that were differentially expressed were identified.

Results: We identified the proteins in 336 spots, which corresponded to 98 different proteins. Highly basic proteins (11.3%) were present in the granule-containing cell fraction, and cytoskeleton/migration related proteins accounted for 24.7% of the total. We detected hyper-acidic forms of cofilin-1, profilin-1 and adenylyl cyclase-associated protein, and hyper-basic forms of eosinophil-derived neurotoxin/eosinophil protein X and major basic protein homolog. Evidence was produced of the tri-glycosylation of the heavy chain of eosinophil peroxidase. The comparative analysis of eosinophils from asymptomatic and allergic subjects showed the over-expression of hsc70, actin-capping protein and hyper-acidic forms of eosinophil peroxidase heavy chain during allergy.

Conclusions: Novel protein forms were detected among cytoskeleton and granule proteins of resting eosinophils. These overexpressions may be linked to an increased motility and cellular antigen-presenting capacity.

This project was funded by the Swedish Society of Medicine, the Faculty of Medicine (Uppsala University, Sweden) and the International Centre for Genetic Engineering and Biotechnology (Trieste, Italy).

POSTER #30

SENSITIZATION INHIBITS SELECTIVE RECRUITMENT OF EOSINOPHILS TO AIRWAYS AFTER OZONE

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Background: A single ozone exposure causes airway hyperreactivity lasting at least three days, but the mechanism changes over that time period. Eosinophils are present in airways one and three days post-ozone, but the role of eosinophils changes between these time points. Previously, we have shown that depleting eosinophils (using anti-IL5 antibody) prevented airway hyperreactivity one day after ozone exposure but makes hyperreactivity worse three days post-ozone. In contrast, depleting eosinophils in sensitized animals prevents hyperreactivity at both time points. Thus, the role of eosinophils in ozone-induced hyperreactivity changes from destructive to beneficial over three days in naïve animals, but remains destructive in sensitized animals.

Objectives: To test whether ozone exposure alters eosinophil populations in the lungs of sensitized and unsensitized guinea pigs.

Methods: Guinea pigs were exposed to either air or 2ppm O_3 for 4 hours. Some guinea pigs were sensitized to ovalbumin 3 weeks before ozone or air exposure. Migration of recently divided eosinophils and other inflammatory cells after a single exposure to O_3 was tracked using 5-bromo-2'-deoxyuridine (BrdU, 50mg/kg, ip) or 5-ethynyl-2'-deoxyuridine (EdU, 50mg/kg, ip). Bronchoalveolar lavage (BAL) was collected 3 days later and eosinophils analyzed by immunocytochemistry in BrdU labeled samples and flow cytometry in EdU labeled samples.

Results: Sensitization significantly increased BrdU- eosinophils (resident cells) in the BAL (from 1 to 2.7 million). O_3 exposure further increased the number of eosinophils in the lungs to 2.7 (nonsensitized) and 5 million (sensitized). However, while in nonsensitized guinea pigs, O_3 induced eosinophillia is comprised of BrdU+ (new) eosinophils, the eosinophil populations in O_3 sensitized lungs are comprised of BrdU- (resident) cells (5.4% BrdU+ in sensitized animals vs 83% BrdU+ in nonsensitized). O_3 exposure only moderately increased percent BrdU+ non-eosinophil leukocytes in nonsensitized animals from 11% to 29%, but not in sensitized animals. Similar results were obtained with EdU label.

Conclusions: O_3 selectively increases recruitment of new eosinophils to the lungs of naïve guinea pigs 3 days after a single exposure. However, in sensitized animals, O_3 only increases resident eosinophils. A new population of eosinophils appears in the lungs of naïve animals 3 days after O_3 and may have a different phenotype than resident eosinophils.

This project was funded by NIH grants HL-55543, HL54659, HL071795, ES01460.

POSTER #31

OZONE CHANGES AIRWAY EOSINOPHILS

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Background: Asthma exacerbations and hospitalizations increase on days with high atmospheric ozone and last for several days. In an animal model of ozone-induced airway hyperreactivity, eosinophils have been highlighted as important inflammatory cells. Immediately after ozone exposure, eosinophils mediate airway hyperreactivity by releasing major basic protein, an antagonist of inhibitory M2 receptors on parasympathetic nerves. Depleting eosinophils prevents airway hyperreactivity one day after ozone exposure. However, depleting eosinophils markedly worsens ozone induced hyperreactivity three days after ozone exposure. This finding indicates the role of eosinophils changes between 1 and 3 days after ozone exposure. Therefore, we tested whether ozone was changing expression of specific eosinophil products.

Objectives: To investigate expression of eosinophil products three days after ozone exposure.

Methods: Guinea pigs were injected with 5-ethynyl-2'- deoxyuridine (EdU 50mg/kg, ip) 30 minutes before air or ozone exposure (2 parts/million, for 4 h) and 2 hours after, and then injected daily. Three days later, animals were killed and bronchoalveolar lavage (BAL) cells were harvested and spun onto slides for immunohistochemical analysis. Eosinophils were identified by chromorop2R staining or by morphology. Cells that had divided after exposure to ozone were identified by positive EdU staining (EdU is incorporated into DNA during division). EdU positive and negative Eosinophils were isolated from the BAL by fluorescence-activated cell sorter and cDNA synthesized for real-time RT-PCR.

Results: The number and percentage of EdU(+) eosinophils were significantly higher in ozone-exposed animals (35%) than those exposed to air (15%). Ozone increased expression of IL-5 receptor and nerve growth factor protein (by immunocytochemistry) on all eosinophils, but the increase was much greater in the newly synthesized EdU(+) eosinophils. Ozone also significantly increased CCR3, EPO, MBP1, and M3 muscarinic receptor expression (by real-time RT-PCR) regardless of whether the eosinophils were EdU(+) or not. Different from all other eosinophil populations, MBP2 expression is significant higher (approximately 40 fold increase) in newly synthesized eosinophils after exposure to ozone. Despite positive antibody staining for nerve growth factor (NGF), there was no detectable nerve growth factor mRNA in BAL eosinophils in any conditions.

Conclusions: These data indicate that the eosinophil populations in the lungs change regarding their age (newly recruited vs resident) and the expressions of eosinophil products change after exposure to ozone. These changes may explain why the contributions of eosinophils to ozone induced hyperreactivity are different between one and three days after ozone exposure.

This project was funded by a grant from NIH Grant: HL55543, ESO1460.

POSTER #32

NATURAL HISTORY OF EOSINOPHILIC GASTROENTERITIS

Guillaume Pineton de Chambrun, Florent Gonzalez, Jean-Yves Canva, Samia Gonzalez, Lucie Houssin, Antoine Cortot and Jean-Frédéric Colombel.

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Background: Eosinophilic gastroenteritis is a rare benign disease characterized by tissue eosinophilic infiltration that may involve several digestive tract layers. The underlying molecular mechanisms predisposing to this disease are unknown.

Objectives: To assess clinical features and long-term outcome of patients with eosinophilic gastroenteritis (EG) followed from 1992 to 2007 in our center.

Methods : In a retrospective study, diagnosis of EG was made according to admitted criteria (Talley, Shorter et al. 1990) after exclusion of other eosinophilic digestive diseases. EG was classified in mucosal, muscular and serosal forms according to Klein (Klein, Hargrove et al. 1970). A follow-up standardized questionnaire was full-filled during clinical consultation and/or contact with the attending physician in order to appreciate the long-term outcome of the disease.

Results: Forty-one patients were diagnosed with EG (27 M et 14 F), with a median age at diagnosis of 38 yrs (16-70). Abdominal pain, diarrhea, nausea and/or vomiting and ascitis were the most frequent revealing symptoms in respectively 70, 48, 45 and 39% of cases. Others inaugural symptoms were dysphagia, rectal bleeding and jaundice. Median time between first symptoms and diagnosis was 1.5 yrs (0,16-27). EG was mucosal, serosal and muscular in 44, 39 and 12 % of cases respectively. Disease location, evaluated by multiples intestinal biopsies from esophagus to colon, was mostly duodenal (63%), ileal (45%) and colonic (66%) and more rarely esophageal (20%) and gastric (32%). Three patients had an eosinophilic duodenopancreatic infiltration presenting as chronic pancreatitis. Blood hyper-eosinophilia (>500/mm^3) was present in 31 patients (79%) associated with a serum IgE increase in 22 (66%). Among the 27 patients treated (66%), more than half (17) received systemic corticosteroids (1mg/kg), 4 Chromoglycate and 6 anti-histaminic therapy. After a median follow-up of 15 years (1-17), 3 different courses were identified: 18 patients (44%), (of whom 9 had a serosal form) presented with a unique flare of the disease without relapse, 14 (36%) presented multiple flares separated by periods of full remission (recurring forms) and 9 (21%) suffered from a chronic continuous form. Three patients were steroid-dependent. No myeloproliferative transformation was observed.

Conclusions: Clinical presentation of EG is heterogeneous depending on its histological form. The evolution of the disease is often benign with no relapse. However more than half of patients presented with a more complex form characterized by unpredictable relapses and a chronic course in some of them.

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Talley, N. J., R. G. Shorter, et al. (1990). "Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues." Gut 31(1): 54-8.

POSTERS: SESSION VIII

Induction/Regulation of Eosinophil-Mediated Damage, Repair, Remodeling and Fibrosis (Posters 33-35)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
33	**Verhein, Kirsten**	**EOSINOPHILS STIMULATE SUBSTANCE P IN GUINEA PIG PARASYMPATHETIC NERVES**	**Kirsten C. Verhein, David B. Jacoby and Allison D. Fryer**
34	**Walsh, Marie-Therese**	**EOSINOPHIL PEROXIDASE INDUCES CELL CYCLE AND GROWTH STIMULATING EFFECTS ON IMR-32 NERVE CELLS**	**Marie-Therese Walsh, Katie Connell, Senan Glynn and Richard W. Costello**
35	**Walsh, Marie-Therese**	**SPHINGOSINE 1-PHOSPHATE AND LYSOPHOSPHATIDIC ACID UP-REGULATE EXPRESSION OF ADHESION MOLECULES AND EOSINOPHIL CHEMOATTRACTANT IN A CHOLINERGIC NERVE CELL LINE**	**Marie-Therese Walsh, Michael Maloney, Mazin Atiyeh and Richard W. Costello**

POSTER #33

EOSINOPHILS STIMULATE SUBSTANCE P IN GUINEA PIG PARASYMPATHETIC NERVES

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Background: A single ozone exposure causes airway hyperreactivity lasting at least three days, but the mechanism changes over that time period. One day after ozone exposure, airway hyperreactivity in guinea pigs is mediated by eosinophil activation and release of major basic protein that inhibits neuronal M_2 receptor function. Three days post-ozone, blocking tachykinins with neurokinin receptor antagonists prevents airway hyperreactivity. Eosinophils are present in airways at both time points and the relationship between eosinophils and tachykinin expression in nerves is unknown.

Objectives: To test whether eosinophils stimulate tachykinin expression in guinea pig parasympathetic nerves using primary nerve cultures.

Methods: Parasympathetic nerves were isolated from guinea pig tracheas and cultured in serum free media on matrigel coated chamber slides. Parasympathetic neurons were enriched using selective plating and treatment with cytosine arabinoside to kill dividing cells. Parasympathetic nerve cell bodies cluster together in culture, and there are typically 5-15 clusters per well on a 4 well chamber slide. Eosinophils, isolated from guinea pig peritoneal lavage, were added to parasympathetic neuronal cultures and incubated for 2 days (200,000-500,000 eosinophils per well). Nerves were fixed with 4% paraformaldehyde and stained for neuronal markers with antibodies to neurofilaments SMI311 (1:500) and SMI312 (1:2000), and for substance P (1:500). Fluorescent secondary antibodies were used to visualize nerves and substance P. Nerves were identified by neurofilament staining and substance P expression quantified using a method adapted from Tuder et al. (2003 AJRCMB 29:88) in which background intensity was subtracted from average fluorescence intensity of substance P in neurofilament labeled neurites.

Results: Parasympathetic nerves cultured with live eosinophils for 2 days have increased production of substance P compared to parasympathetic nerves cultured without eosinophils (1.9 ± 0.4 fold increase). Adding nerve growth factor (NGF) to cultures for 4 days slightly increased substance P (1.4 ± 0.05 fold increase). However, blocking NGF with an antibody did not prevent the eosinophil-induced increase in substance P.

Conclusions: Eosinophils increase substance P production by parasympathetic nerves by a mechanism that is not dependent on NGF.

This project was funded by a grant from the American Heart Association 0810148Z (KCV) and NIH grants HL-55543, HL54659, HL071795, ES01460

POSTER #34

EOSINOPHIL PEROXIDASE INDUCES CELL CYCLE AND GROWTH STIMULATING EFFECTS ON IMR-32 NERVE CELLS

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Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Background: Eosinophils exert many of their inflammatory effects in allergic disorders by degranulation and release of cationic granule proteins including eosinophil peroxidase (EPO). In sub-cytotoxic concentrations, eosinophil granule proteins increase transcriptional expression of various growth factors in airway cells.

Objectives: The purpose of this study was to determine the effects of sub-cytotoxic concentrations of eosinophil peroxidase (EPO) on expression and sub-cellular localisation of the linked cell growth and cell cycle mediators, focal adhesion kinase (FAK), cyclin-dependent kinase inhibitor $p27^{kip}$ and the epidermal growth factor receptors HER1 and HER2.

Methods: The methods used were real-time PCR, Western blotting of membrane, nuclear and cytoplasmic cell fractions and immunoprecipitation.

Results: EPO induced a concomitant time-dependent egress of FAK and $p27^{kip}$ from the cell nucleus to the cytoplasm. Immunoprecipitation indicated a physical association between FAK and $p27^{kip}$, implying that FAK acts as a nuclear-cytoplasmic shuttle for $p27^{kip}$. EPO also induced up-regulation of expression of HER1 and HER2.

Conclusions: Our results imply that EPO potentially induces cell proliferation, by up-regulating HER1 and HER2 and cell cycle, by driving $p27^{kip}$ from the nucleus. This has implication for the role of eosinophils in tissue remodelling and turnover in conditions from asthma to cancer.

This project was funded by grants from the Wellcome Trust and the Health Research Board, Ireland

POSTER #35

SPHINGOSINE 1-PHOSPHATE AND LYSOPHOSPHATIDIC ACID UP-REGULATE EXPRESSION OF ADHESION MOLECULES AND EOSINOPHIL CHEMOATTRACTANT IN A CHOLINERGIC NERVE CELL LINE

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Background: The bioactive lysophospholipids sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPA) act mainly via G-protein coupled receptors $S1P_{1-5}$ and LPA_{1-3} respectively. They also impact on neuronal function and activity and neuronal cell intracellular signalling, including cytoskeletal reorganisation, acetylcoholine release, apoptosis and neurite outgrowth or retraction. They have also been implicated in allergy. Eosinophils accumulate at innervating cholinergic nerves in fatal asthma and in animal models of asthma and adhere to nerve cells in culture via intercellular adhesion molecule-1 (ICAM-1).

Objectives: The purpose of this study was to determine if S1P and/or LPA induce up-regulation of adhesion molecules and eosinophil chemoattractants in an *in vitro* cholinergic nerve cell model, IMR-32 cells, and if the eosinophil granule protein eosinophil peroxidise (EPO) could influence this process.

Methods: The methods used were real-time PCR and Western blotting of nuclear and cytoplasmic cell fractions.

Results: $S1P_1$, $S1P_3$, LPA_1, LPA_2 and LPA_3 were expressed on IMR-32 cells. Both S1P and LPA induced ERK phosphorylation and ERK- and G_i-dependent up-regulation of ICAM-1 expression in IMR-32 cells, with differing time courses. LPA also induced ERK- and G_i-dependent up-regulation of the eosinophil chemoattractant, CCL-26. EPO induced ERK-dependent up-regulation of transcription of $S1P_1$, LPA_1, LPA_2 and LPA_3.

Conclusions: Thus S1P and LPA, acting via G_i-coupled nerve cell receptors, induce up-regulation of adhesion molecules and chemoattractants which stimulate eosinophil accumulation and adhesion to cholinergic nerve cells. In turn, EPO induces up-regulation of S1P and LPA receptors, potentially perpetuating S1P- and LPA- induced effects.

This project was funded by grants from the Wellcome Trust and the Health Research Board, Ireland

POSTERS: SESSION IX

Eosinophils and Disease (II): Malignancy, Parasitosis, and others (Posters 36-44)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
36	**Butterfield, Joseph**	***BABESIA MICROTI* IN EOSINOPHILIC DISORDERS-PRELIMINARY REPORT**	**Joseph H. Butterfield**
37	**Cadman, Emma**	**THE ROLE OF EOSINOPHILS AND MACROPHAGES IN THE IMMUNE RESPONSE TO *BRUGIA MALAYI* INFECTION IN THE LUNG**	**Cadman ET and Lawrence, RA**
38	**Carlson, Marie**	**EOSINOPHIL ACTIVATION IN TWINS, REVEALING A POSSIBLE PATHOPHYSIOLOGICAL MECHANISM IN INFLAMMATORY BOWEL DISEASE?**	**Jonas Halfvarson, Christer Peterson, Anders Gustavsson, Ingrid Stolt, Johan Bohr, Curt Tysk, Lennart Bodin, Marie Carlson**
39	**Johnsson, Marianne**	**EOSINOPHILS EXHIBIT DISTINCT IMMUNOPHENOTYPIC PROFILES IN ACUTE VERSUS CHRONIC GRAFT-VERSUS-HOST DISEASE**	**Marianne Johnsson, Julia Cromvik Stefan Jacobsson, Krista Vaht, Lars Hynsjö, Samuel Lundin, Christine Wennerås**
40	**Lampinen, Maria**	**BUDESONIDE TREATMENT OF PATIENTS WITH COLLAGENOUS COLITIS RESTORES NORMAL EOSINOPHIL ACTIVITY IN THE COLON**	**Maria Lampinen, Michael Wagner, Per Sangfelt and Marie Carlson**
41	**Legrand, Fanny**	**MOLECULAR MECHANISMS OF EOSINOPHIL TUMORICIDAL ACTIVITY**	**Fanny Legrand, Virginie Driss, Sylvie Loiseau, Marie Delbeke, Jean-Emmanuel Kahn, Lionel Prin, Monique Capron**
42	**Lind, Alexandra**	**TISSUE EOSINOPHILIA IN BARRETT ESOPHAGUS AND REFLUX ESOPHAGITIS**	**Alexandra Lind, Peter D. Siersema and Leo Koenderman**
43	**Masterson, Joanne**	**EOSINOPHILIC ILEITIS AND TISSUE REMODELING IN SAMP1 MICE**	**Joanne C. Masterson, Eoin N. McNamee, Joanna Grenawalt, Adrianne Burgess, Zachary D. Robinson, Paul Jedlicka, James J. Lee, Sophie Fillon, Jesus Rivera-Nieves, Glenn T. Furuta**
44	**Cancelled**	**CANCELLED**	

POSTER PRESENTATIONS SESSION IX

POSTER #36

BABESIA MICROTI IN EOSINOPHILIC DISORDERS-PRELIMINARY REPORT

Joseph H. Butterfield

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Background: In 2001 the first successful use of imatinib mesylate for hypereosinophilic syndrome (HES) was self-reported by a patient who subsequently was found to be positive for the PDGFRα/FIP1L1 fusion. Despite discontinuing imatinib after one year his eosinophil count remained normal for the next 5 years; however he continued to be plagued by intractable headaches, paresthesias of the calves and feet, fatigue and mild neuropsychiatric symptoms. Because several family members tested positive for *B microti* an empiric trial for *babesiosis* was given to this patient. These treatments resulted in subsequent improvement in his ongoing symptoms although serologic and PCR evidence for infestation in his case was absent. Based on his experience, the possibility was raised that *Babesia* could be an overlooked trigger for HES and many associated symptoms (Schaller JL, Burkland GA Medscape General Medicine. 2007; 9: 38.).

Objectives: The objective of this study was to determine if patients with eosinophilic disorders show evidence of *B microti* infection.

Methods: Patients referred to the Division of Allergic Diseases during the past year have been tested for *B microti* infestation by serologic assay and/or by rapid PCR in the course of investigation for the possible diagnosis of HES.

Results: Twenty patients with the following eventual causes of eosinophilia were tested for evidence of *B microti* infection during the course of their workups [HES-7; eosinophilia associated with rheumatoid arthritis-1; parasitism-2; Churg Strauss syndrome-1; eosinophilia associated with dermatitis-2; eosinophilic pericarditis-1; eosinophilic pneumonia-2; necrotizing lymphadenitis with eosinophilia-1; eosinophilic myositis-1; eosinophilia with severe episodic vomiting-1; eosinophilia-unknown cause-1]. Fourteen females and six males were seen, ranging in age from 1 to 85 years. In no patient was there serologic or PCR evidence for *B microti* infection. None of the patients were CHIC-2 positive. In the two patients with parasitism (trichinosis, toxocariasis) appropriate treatment resulted in normalization of eosinophilia.

Conclusions: Protozoan infestations with intraerythrocytic parasites including *Babesia* are not commonly associated with hypereosinophilic conditions. The clinical improvement in the index CHIC-2 positive, imatinib-responsive hypereosinophilic patient following treatment for *Babesia*, prompted this ongoing survey for evidence of *B microti*. In this initial series of patients with eosinophilic disorders there is no evidence for infection by *B microti*.

POSTER #37

THE ROLE OF EOSINOPHILS AND MACROPHAGES IN THE IMMUNE RESPONSE TO *BRUGIA MALAYI* INFECTION IN THE LUNG

Cadman ET[1] and Lawrence, RA[1]

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Background: We have previously shown that eosinophils are required for the clearance of primary, but not secondary, infection with the nematode parasite *Brugia malayi*. The eosinophil granule protein eosinophil peroxidase (EPO) has also been shown to play a role in clearance of helminth parasites, and lack of eosinophils has been shown to influence the development of airway hyperresponsiveness during *Brugia malayi* infection.

Objectives: To investigate the the roles of eosinophil peroxidase, regulatory T and B cells and macrophages in the immune response and development of airway hyperresponsiveness during *Brugia malayi* infection

Methods: Mice were immunised 3 times with 200mg Mf antigen prior to challenge in a "secondary" infection. Immunised and non-immunised mice were challenged with 200,000 Mf intravenously. Lung function was measured by whole body plethysmography. Infiltration of cells into the lung was measured by cytospin of bronchoalveolar lavage. Lung structure was investigated by histology. Serum antibodies, bronchoalveolar lavage cytokines and cytokines from splenocyte culture were analysed by ELISA.

Results: Lack of macrophages and EPO increases airway hyperresponsiveness during primary infection. Lack of macrophages and B cells also slows parasite clearance. Lack of regulatory T cells does not affect parasite clearance or airway hyperresponsiveness, but does result in the premature termination of the anti-parasite IgG1 response.

Conclusions: Eosinophil granule proteins, regulatory T cells, B cells and macrophages all influence the immune response to *Brugia malayi* infection. These effects are subtle and further work will be required to fully characterise these effects and the mechanism behind them.

This project was funded by a grant from the Biotechnology and Biological Sciencees Research Council

POSTER #38

EOSINOPHIL ACTIVATION IN TWINS, REVEALING A POSSIBLE PATHOPHYSIOLOGICAL MECHANISM IN INFLAMMATORY BOWEL DISEASE?

Jonas Halfvarson[1], Christer Peterson[2], Anders Gustavsson[1], Ingrid Stolt[2], Johan Bohr[1], Curt Tysk[1], Lennart Bodin[3], Marie Carlson[2].

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Background: Fecal calprotectin (FC) is a marker of neutrophil and monocyte activity and is as well as eosinophil fecal marker eosinophil protein X (EPX) and eosinophil cationic protein (ECP) elevated in inflammatory bowel disease[1]. Elevated FC has also been observed in first-degree relatives of patients with inflammatory bowel disease[2,3]. Increased NfκB activity has been demonstrated in healthy co-twins to twins with inflammatory bowel disease. These data indicate a low degree of inflammatory activity in first-degree relatives, but whether the eosinophils are involved is unknown.

Objectives: Our aim was to study the degree of eosinophil activity in healthy co-twins to twins with inflammatory bowel disease.

Methods: Twins born 1936-1986 from a Swedish population based cohort of twins with inflammatory bowel disease were clinically phenotyped using the Montreal classification. A previously described control group (n=44) was used as reference population[1]. EPX and ECP were measured by ELISA kit and UniCAP ®, respectively. Normal levels for EPX and ECP was ≤ 2.15 and ≤ 5.81 μg/g, respectively. Logistic regression was used, and odds ratios (OR) with corresponding 95% confidence intervals (95% CI) were calculated.

Results: 109 twins, who had not undergone extensive Crohn's disease (CD) or ulcerative colitis (UC) related resections, participated. 33 twin pairs with CD (concordant monozygotic n=6, discordant monozygotic n=13, concordant dizygotic n=l and discordant dizygotic n=13), 16 twin pairs with UC (concordant monozygotic n=1, discordant monozygotic n=10 and discordant dizygotic n=5) as well as 11 single twins (CD n=3, UC n=3, healthy co-twins to twins with CD n=3 and healthy co-twins to twins with UC n=2) participated. Elevated levels of EPX and ECP were detected in twins with CD [OR 16.6 (4.3-96.6) and 5.2 (1.3-31.1), respectively] and in twins with UC elevated levels of EPX [OR 5.7 (1.0-39.7)], whereas healthy co-twins demonstrated normal levels of fecal EPX and ECP.

Conclusions: Eosinophils, as opposed to neutrophils, are not activated in healthy co-twins to twins with inflammatory bowel disease. In contrast, twins with IBD have activated eosinophils in the intestinal mucosa. These data suggest a possible role for the eosinophil in the development of manifest inflammation in patients with IBD.

This project was funded by a grant from the Swedish Research Council – Medicine, The Ihre Foundation and the Hedlund Foundation.

POSTER #39

EOSINOPHILS EXHIBIT DISTINCT IMMUNOPHENOTYPIC PROFILES IN ACUTE VERSUS CHRONIC GRAFT-VERSUS-HOST DISEASE

Marianne Johnsson[1], Julia Cromvik[2] Stefan Jacobsson[3], Krista Vaht[2], Lars Hynsjö[3], Samuel Lundin[4], Christine Wennerås[1,2]

[1]Department of Clinical Bacteriology, [2]Department of Hematology and Coagulation, [3]Department of Clinical Chemistry and [4]Department of Microbiology and Immunology, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden.

Background: Graft-versus-host disease (GvHD) is a common complication of allogeneic hematopoietic stem cell transplantation, which is an established treatment for haematological malignancies. GvHD diagnosis often requires biopsy from afflicted tissue such as skin or intestine. Eosinophilia has been reported to precede the development of both acute and chronic GvHD, and intestinal infiltration of eosinophils correlates with the gravity of acute GvHD. Eosinophilia has been shown to be associated with a milder form of chronic GvHD.

Objectives: To develop a diagnostic tool based on the immunophenotypic profile of blood eosinophils to aid in the diagnosis of acute and chronic GvHD.

Methods: EDTA blood from both healthy donors (n=10) and adult hematopoietic stem cell transplant recipients (n=27) was collected. Absolute numbers of eosinophils were determined in a cell counter. Plasma was collected for later analysis of eosinophil chemoattractants using Cytometric Bead Array. Unfractionated leucocytes were incubated with panels of monoclonal antibodies (n=24) conjugated with three different fluorochromes, against both intra- and extracellular molecules. All data was analyzed using the multivariate technique of pattern recognition Projections to Latent Structures (PLS-SIMCA).

Results: Multivariate analysis revealed that patients with acute GvHD formed a separate cluster from those without GvHD or chronic GvHD. The immunophenotypic eosinophil profile which characterized patients with acute GvHD was high extracellular expression of CD16, CD69, CCR3, CD23, CD54 and high intracellular expression of TNF and IL-4. Chronic GvHD featured enhanced expression of CD18, CD11c, $CRTH_2$, FPRL-1, CD44 and CD9, compared to transplanted patients without GvHD.

Conclusions: Eosinophils display diverse immunophenotypic profiles in patients with acute and chronic GvHD, respectively. Hopefully this can be exploited to facilitate the diagnosis of GvHD.

This project was funded by grants from the Swedish Research Council, Cancer and Allergy Foundation, ALF Funding, Västra Götaland Region Research Funding and the Blood Cancer Foundation.

POSTER #40

BUDESONIDE TREATMENT OF PATIENTS WITH COLLAGENOUS COLITIS RESTORES NORMAL EOSINOPHIL ACTIVITY IN THE COLON

Maria Lampinen[1], Michael Wagner[1], Per Sangfelt[1] and Marie Carlson[1]

[1] Department of Medical Sciences, Gastroenterology research group, University Hospital, Uppsala, Sweden.

Background: Collagenous colitis (CC), a subgroup of the inflammatory bowel diseases, is characterized by a thick subepithelial collagen layer in the colon in the presence of chronic watery diarrhea and macroscopically normal colonic mucosa. The pathogenesis is unknown, but we have previously found a low grade eosinophil activity in colonic perfusion fluid from CC patients, and others have shown increased numbers of $CD4^+$ T cells in lamina propria and $CD8^+$ T cells in the epithelium.

Objectives: The aim of this study was to evaluate the activity of colonic eosinophils, neutrophils and T cells in active and quiescent CC, in comparison with healthy controls.

Methods: Eleven patients with active CC were studied before and after 8 weeks of steroid treatment (Budesonide, 9 mg daily). On both occasions, ileo-colonoscopy was performed, and biopsy samples were taken. Ten healthy controls underwent ileo-colonoscopy on one occasion. Cell suspensions were prepared and analysed by flow cytometry. Eosinophils with a high surface expression of CD44 and low CD9 expression were classified as activated, and CD69 was used as an activation marker for T cells. Neutrophil activity was assessed by their expression of CD66b.

Results: All patients responded to the treatment; ten out of eleven went into complete remission. The eosinophils in active CC showed significantly increased activity measured as CD9 expression, and a tendency to increased CD44 expression compared to controls. The activity was normalized after treatment, with lower CD44 and higher CD9 expression. CD8+ T cells from untreated CC patients had a lower CD69 expression than controls, and a tendency of lower CD69 was observed on CD4+ T cells before treatment. After steroid treatment, the levels of CD69 were increased on both types of T cells and were not significantly different from controls. Neutrophils were not activated in CC patients before or after treatment compared to control subjects.

Conclusions: The inflammation in CC is characterized by activated eosinophils, but in contrast to ulcerative colitis, there is no infiltration of activated neutrophils. CD4+ and CD8+ T cells are present in increased numbers during active CC, but with a lower grade of activity than in the healthy colon. Steroid treatment restores normal activation of eosinophils and, to a certain extent, T cells and induces clinical remission.

This project was funded by grants from Swedish Research Council– Medicine, the Hedlund Foundation and the Ihre Foundation

POSTER #41

MOLECULAR MECHANISMS OF EOSINOPHIL TUMORICIDAL ACTIVITY

Fanny Legrand[1], Virginie Driss[1], Sylvie Loiseau[1], Marie Delbeke[1], Jean-Emmanuel Kahn[2], Lionel Prin[2], Monique Capron[1].

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Background: Eosinophils, classically involved in helminth parasitic infections and allergic diseases, have been described as inflammatory leukocyte infiltrate during many cancers. Eosinophilia named TATE for "tumor associated tissue eosinophilia" may have favourable prognostic value in some carcinoma, notably in colorectal cancers. Some epidemiological studies have been performed to correlate TATE and cancer prognosis, however little is known on the exact role played by eosinophils in antitumor responses. Although eosinophils can be attracted at tumor sites, their direct interactions with tumor cells have not been extensively investigated.

Objectives: To evaluate the *in vitro* tumoricidal activity of human eosinophils towards a colorectal adenocarcinoma tumor cell line, the Colo-205 and to investigate the molecular mechanisms involved in eosinophil mediated cytotoxicity.

Methods: Peripheral blood eosinophils were purified from normal and eosinophilic donors by immunomagnetic negative selection. Eosinophil chemotaxis towards Colo-205 was measured by Transwell assay. Different flow cytometry protocols were performed for measurement of eosinophil adhesion to tumor cells and of target cell death, PKH-26-Annexin V-FITC double stainings and FATAL assay. Cytokine and mediator release was evaluated by ELISA. Interplay of different receptors and mediators were studied using respectively specific neutralizing antibodies and inhibitors.

Results: Our results showed that human eosinophils are attracted by Colo-205 cells, are able to induce contact dependent apoptosis and necrosis of tumor cells. Eosinophil specific mediator release like ECP and EDN was detected after incubation with tumor cells. Screening of expression by eosinophils of perforine, granulysine and granzyme family members, using RT-PCR and flow cytometry approaches revealed that eosinophils express granzyme A. Granzyme A was released by eosinophils incubated with Colo-205 and FUT-175, a highly specific granzyme inhibitor, inhibited tumor target cell death suggesting a role for this mediator in tumoricidal effect of eosinophils towards the colorectal carcinoma cell line.

Conclusions: This work provides evidence for eosinophil tumoricidal activity towards Colo-205 cell line and suggests involvement of both eosinophil and T cell specific mediators. A better understanding of the role of eosinophils in tumor development might have important therapeutic implications.

This project was funded in part by grants from ANR and Nord-Pas-de-Calais Region

POSTER #42

TISSUE EOSINOPHILIA IN BARRETT ESOPHAGUS AND REFLUX ESOPHAGITIS

Alexandra Lind[1,2], Peter D. Siersema[1] and Leo Koenderman[2]

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Background: Barrett Esophagus is a metaplastic lesion of esophagis in which squamous stratified epithelium is replaced by intestinal type columnar epithelium (Barrett esophagus (BE)). This epithelial switch is thought to be a consequence of continuous exposure to reflux components: acid and bile salts. In BE, Th2-effector cells such as mast cells and eosinophils are found (Moons et al, 2005). In contrast, reflux esophagitis (RE), which is preceding BE, is thought to be characterized by a predominantly Th1 response (Fitzgerald et al, 2002). Interestingly, healthy esophagus is devoid of eosinophils which is in contrast to intestinal tissue where presence of eosinophils is common. Eosinophils are also found in esophagus tissue in gastroesophageal reflux disease (GERD) and RE (Rodrigo et al, 2008). As eosinophils have cytotoxic and tissue remodeling properties, we studied their presence and phenotype in BE and RE.

Objectives: To compare the number and morphology of eosinophils in RE and BE.

Methods: Biopsies obtained for diagnostic purposes during esophagoscopy were formalin-fixed and embedded in paraffin. Biopsies were obtained from 10 BE patients and 11 RE patients. The number of eosinophils was measured by immunohistochemical staining for Major Basic Protein (MBP). The number and morphology of eosinophils was determined by image analysis of images obtained by light microscopy. Two areas: epithelium and lamina propria were assessed separately. A score was given to assess the number of eosinophils and the presence of free granules.

Results: In nine of 10 BE patients moderate to high numbers of MBP-positive cells were found in the lamina propria (LP). These cells had a non-activated phenotype and no free granules were found. In the epithelium of these BE patients, no eosinophils or free MBP-granules were present. In RE biopsies, eosinophils were present in the lamina propria as well. However in contrast to BE where only non-activated eosinophils were found, eosinophils in RE had an activated, degranulating phenotype. In addition, the amount of free MBP granules was in BE biopsies low compared to RE biopsies. In 4 of 11 RE biopsies, eosinophils and free MBP granules were also present in the epithelium. RE patients with a more profound inflammatory reaction had higher amount of free granules and activated eosinophils in the epithelium.

Conclusions: The mechanism underlying the difference in phenotype of eosinophils in BE and RE is unclear. However, it is tempting to speculate that eosinophilia in BE can be explained by homeostatic mechanism normally operating in intestinal tissue, whereas eosinophils in RE are attracted by factors secreted by damaged squamous epithelial cells.

This project was funded by a grant from Dutch foundation of Gastroenterology and Hepatology (nr. WO 06-26)

POSTER #43

EOSINOPHILIC ILEITIS AND TISSUE REMODELING IN SAMP1 MICE

Joanne C. Masterson[1,2,5], Eoin N. McNamee[2,3,5], Joanna Grenawalt[1,2,5], Adrianne Burgess[1,2,5], Zachary D. Robinson [1,2,5] , Paul Jedlicka[4,5], James J. Lee[6], Sophie Fillon[1,2,5], Jesus Rivera-Nieves[2,3,5], Glenn T. Furuta[1,2,5]

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Background: The senescence-accelerated (SAMP1) mouse strain develops ileitis and recapitulates the pathology of human Crohn's disease. Further examination of ileal tissue from this strain reveals significant eosinophilic infiltration and remodeling, similar to that observed in eosinophilic gastrointestinal diseases (EGIDs).

Objectives: We hypothesize that eosinophilic infiltration contributes to intestinal tissue remodeling. The aim of this study was to characterize tissue remodeling, permeability and eosinophilic infiltration in the SAMP1 spontaneous model of Crohn's-like ileitis.

Methods: A time-course and histological features of ileitis in SAMP1 mice were assessed using H&E staining and MBP immunohistochemistry. Intestinal permeability was measured by the FITC labeled Dextran gavage method. Real Time PCR analysis was performed on ileal tissue for the TGF-β family, epithelial and mesenchymal markers of remodeling, collagen and extracellular matrix components, remodeling associated proteases, eotaxins, their receptors and mucin genes. All assessments were compared to age-matched control AKR mice. Eosinophil inhibition studies were performed with α-CCR3 and α-IL-5 antibody infusions.

Results: SAMP1 mice demonstrated increased ileal inflammation and tissue remodeling (collagen deposition, muscularis propria and goblet cell hyperplasia, villous blunting) from 10 to 50 weeks-of-age. These findings were not observed in AKR control mice. Major basic protein staining revealed an increase in intact eosinophils beginning at 10 weeks-of-age (>30 per HPF, 10X) that reached a peak at 30 weeks-of-age (~90 per HPF, 10X). Eosinophilic infiltration was noted both in the mucosa and submucosal layers. At 10 weeks-of-age eosinophil degranulation was identified and persisted through to 20 weeks The fibroblast chemo-attractant fibronectin was the most increased gene (20 fold $p<0.0001$-20 weeks, 30 fold $p=0.0007$-40 weeks compared to age matched AKR). Intestinal permeability in SAMP1 was significantly greater than AKR mice beginning at 20 weeks-of-age, a finding that increased through 50 weeks (2 fold p=0.05-20wks, 4 fold p=0.01-30wks, 5.5 fold p=0.0002-40wks compared to age matched AKR). a-CCR3 and a-IL-5 infusions lead to decreased eosinophilic infiltration ($p<0.05$) and total inflammation ($p=0.005$). Fibronectin gene expression decreased in treated vs. isotype control mice (0.3 fold $p=0.002$).

Conclusions: SAMP1 mice demonstrate histological and molecular features of eosinophilic infiltration and tissue remodeling. Reduction of eosinophilic infiltration leads to the reduction of overall inflammation and remodeling in this mouse model. We anticipate that this model will provide a valuable tool for further elucidating the eosinophil's role in the pathogenesis of inflammatory bowel and eosinophilic gastrointestinal diseases.

This project was funded by a grant from NIH RO1 DK 62245.

POSTERS: SESSION X

Existing Treatment Strategies and Targets for Eosinophil-Mediated Disease (Posters 45-46)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
45	**Ackermann, Felix**	**HYPEREOSINOPHILIC SYNDROMES : PROGNOSIS IMPROVEMENT IN A NEW THERAPEUTIC ERA**	**Felix Ackermann, Pierre Charles, Catherine Grandpeix-Guyodo, Ibrahim Marroun, Anne-Marie Piette, Olivier Bletry, Jean-Emmanuel Kahn**
46	**Straumann, Alex**	**BUDESONIDE FOR ADOLESCENTS AND ADULTS WITH ACTIVE EOSINOPHILIC ESOPHAGITIS**	**Alex Straumann, Sebastien Conus, Lukas Degen, Stephanie Felder,‡ Mirijam Kummer, Hansjürg Engel, Christian Bussmann, Christoph Beglinger, Alain Schoepfer, and Hans-Uwe Simon**

POSTER #45

HYPEREOSINOPHILIC SYNDROMES : PROGNOSIS IMPROVEMENT IN A NEW THERAPEUTIC ERA

Felix Ackermann[1], Pierre Charles[1], Catherine Grandpeix-Guyodo[1], Ibrahim Marroun[1], Anne-Marie Piette[1], Olivier Bletry[1], Jean-Emmanuel Kahn,[1,2].

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Background: Hypereosinophilic Syndrome (HES) is a rare and heterogeneous group of disorders, now divided in 3 subtypes: myeloproliferative (M-HES), lymphocytic (L-HES) and idiopathic (I-HES). New targeted molecules became recently available, like tyrosine-kinase inhibitors (ITK) or anti IL-5 antibodies, which have greatly modified HES treatment. However, there is no data about the impact of these new therapies on the long-term prognosis of HES, which was considered poor, based on series published before 1990.

Objectives: To describe HES long-term prognosis according to the variant and treatment.

Methods: Our monocentric retrospective study analyses the prognosis of 49 patients with HES, according to the recent molecular classification proposed by Klion *and al.* on behalf of the HES Working Group: 25 men and 24 women were followed for a mean period of 8,2 years (± 5,5 years). The mean age at diagnosis was 46.5 y (± 14.6). Nine patients (18 %) were considered as M-HES among them 6 were FIP1L1-PDGFRA (F/P) positive. Twelves patients (24 %) had L-HES and 28 (58 %) were classified as I-HES.

Results: Clinical manifestations mainly involved skin (51%), gastrointestinal tract (42%) and heart in only 6 patients (12 %). All the M-HES patients received imatinib mesylate, successfully for 7 (6 FP-positive and 1 FP-negative). Thirty-one patients with L-HES or I-HES were treated with corticosteroids and 17 patients are currently treated with mepolizumab. The overall estimated survival is 100 %, 87.5 % and 66 % at respectively 5, 10 and 15 years. Two F/P negative M-HES patients died: one following acute leukemia, the second of an unrelated cardiac event, respectively 77 and 180 months after diagnosis. A woman in the L-HES group died of an unexplained nasal massive haemorrhage at 177 months. No patient with L-HES developed T cell lymphoma during a mean following period of 9,6 years (± 4,6 years). All the 28 patients with idiopathic variant are alive.

Conclusions: The only available data concerning prognosis of HES (Chusid *and al*.,1975, Lefebvre *and al*., 1989) were published for more than 20 years and suggested a poor prognosis. In the French cohort reported in 1989 (40 patients), survival rate was 80% at 5 years and 42% at 10 and 15 years. Most of deaths were attributed to cardiac involvement. We observed, in our cohort, a great improvement of survival, which may be explained by a lower frequency of cardiac involvement but also of patients classified as M-HES, usually associated with the worst prognosis. Furthermore, the presence of F/P is now predictive of complete hematologic response with imatinib therapy, resulting in a very good long-term prognosis. In conclusion, prognosis of HES markley improved in the 2 past decades, with a decrease of cardiac related deaths. Our study also suggests that FP-negative M-HES patients, resistant to ITK, are at higher risk.

POSTER #46

BUDESONIDE FOR ADOLESCENTS AND ADULTS WITH ACTIVE EOSINOPHILIC ESOPHAGITIS

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Background & Aims: Eosinophilic esophagitis (EoE) is a rapidly increasing, chronic-inflammatory disease of the esophagus characterized by esophageal symptoms and a dense tissue eosinophilia, both refractory to proton pump inhibitors. Topical corticosteroids have proven effective in pediatric EoE. In contrast, no controlled study with corticosteroids has been reported in adult EoE.

Methods: A randomized, double-blind, placebo-controlled trial evaluated the effect of twice daily 1 mg swallowed budesonide in adolescent and adult patients with active EoE within a timeframe of 15 days. Pre- and post-treatment disease activity was assessed clinically, endoscopically, and histologically. The primary end-point was the reduction in the mean eosinophil number/hpf (=esophageal eosinophil load) in the esophageal epithelium. Furthermore, esophageal biopsies and blood were analyzed using immunofluorescence and immunoassays, respectively, to test potential biomarkers for their suitability in monitoring inflammation and predicting treatment responses.

Results: A 15-day therapy evoked a highly significant decrease in the eosinophil load in the budesonide group (n=18) (from 68.2 to 5.5 eosinophils/hpf; $p<0.0001$), but not in the placebo group (n=18) (from 62.3 to 56.5 eosinophils/hpf, $p=0.48$). Clinically, both frequency ($p=0.0050$) and intensity ($p=0.0315$) of dysphagia significantly improved in the budesonide compared with the placebo group. Endoscopically, we observed that white exudates and red furrows were reversible. Budesonide, but not placebo, treatment reduced epithelial cell apoptosis and molecular remodeling events in the esophagus. No serious adverse events occurred.

Conclusions: Short-term treatment with budesonide is highly effective in reducing eosinophilic inflammation and symptoms of active EoE in adolescent and adult patients.

POSTER DISCUSSION SESSION XI

New Therapeutic Targets for Eosinophil-mediated Disease (Posters 47-48)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
47	**Cancelled**	**CANCELLED**	
48	**Tanigai, Tomomi**	**DOCOSAHEXAENOIC ACID ATENUATES EOSINOPHIL FUNCTIONS**	**Tomomi Tanigai, Shigeharu Ueki, Tomoko Masuda, Hikari Kato, Junko Kihara, Masahide Takeda, Wataru Ito, Hiroyuki Kayaba, Ken Ohta and Junichi Chihara**

POSTER #48

DOCOSAHEXAENOIC ACID ATENUATES EOSINOPHIL FUNCTIONS

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Background: One explanation of the recent dramatic increase in number of allergic disease is a result of changes in the environment and eating habits. Particularly, involvement of western style-habitual food intake is suggested to play a role. Several studies have shown that fish oil-enriched diets attenuate the progression of human and experimental allergic disorders, including asthma. Accumulating evidences have indicated that n-3 polyunsaturated fatty acids (PUFA) from fish attenuate immune cell response. Recently, several studies have been shown the anti-inflammatory effects of n-3 PUFA were mediated through Peroxisome proliferators activated receptors (PPARs). Until now, relatively little is known about the effect of PUFA on human eosinophil functions.

Objectives: To clarify the direct effect of PUFA on eosinophil functions, the effect of docosahexaenoic acid (DHA; n-3 PUFA) and linoleic acid (LA; n-6 PUFA) on eosinophil survival, adhesion, and chemotaxis were studied in vitro systems.

Methods: Peripheral blood eosinophils were purified by negative selection using anti-CD16 immunomagnetic beads and a MACS system. We investigated the effect of DHA and LA on following eosinophil functions. Eosinophil survival was determined using flow cytometer by staining with Annexin V and propidium iodine. Eosinophil adhesion to soluble ICAM-1 coated plates was assessed by counting adherent eosinophils using light microscopy. Migration of eosinophils was conducted in duplicate using 5-μm pore-size polycarbonate membranes in Boyden chambers. Changes in intracellular free calcium levels were detected as the increase in fluorescence intensity of calcium-sensitive dye fura-2.

Results: We found that eosinophil survival was inhibited by DHA in a dose- and time-dependent manner. A tendency toward an increase in caspase-3 activity was also observed in eosinophils cultured with DHA. However, PPARα or PPARγ antagonist failed to reverse the pro-apoptotic effect of DHA. DHA attenuated the eotaxin-induced Ca2+ influx, adhesion, and chemotactic activity. In contrast, these inhibitory effects were not observed by LA.

Conclusions: Our results raise the possibility of n-3 PUFA as an inhibitor of the heightened eosinophil response. Our study also supports the importance of n-3 PUFA-rich diet as a therapeutic strategy to treat asthma and other allergic inflammatory diseases.

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