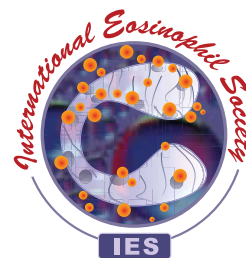
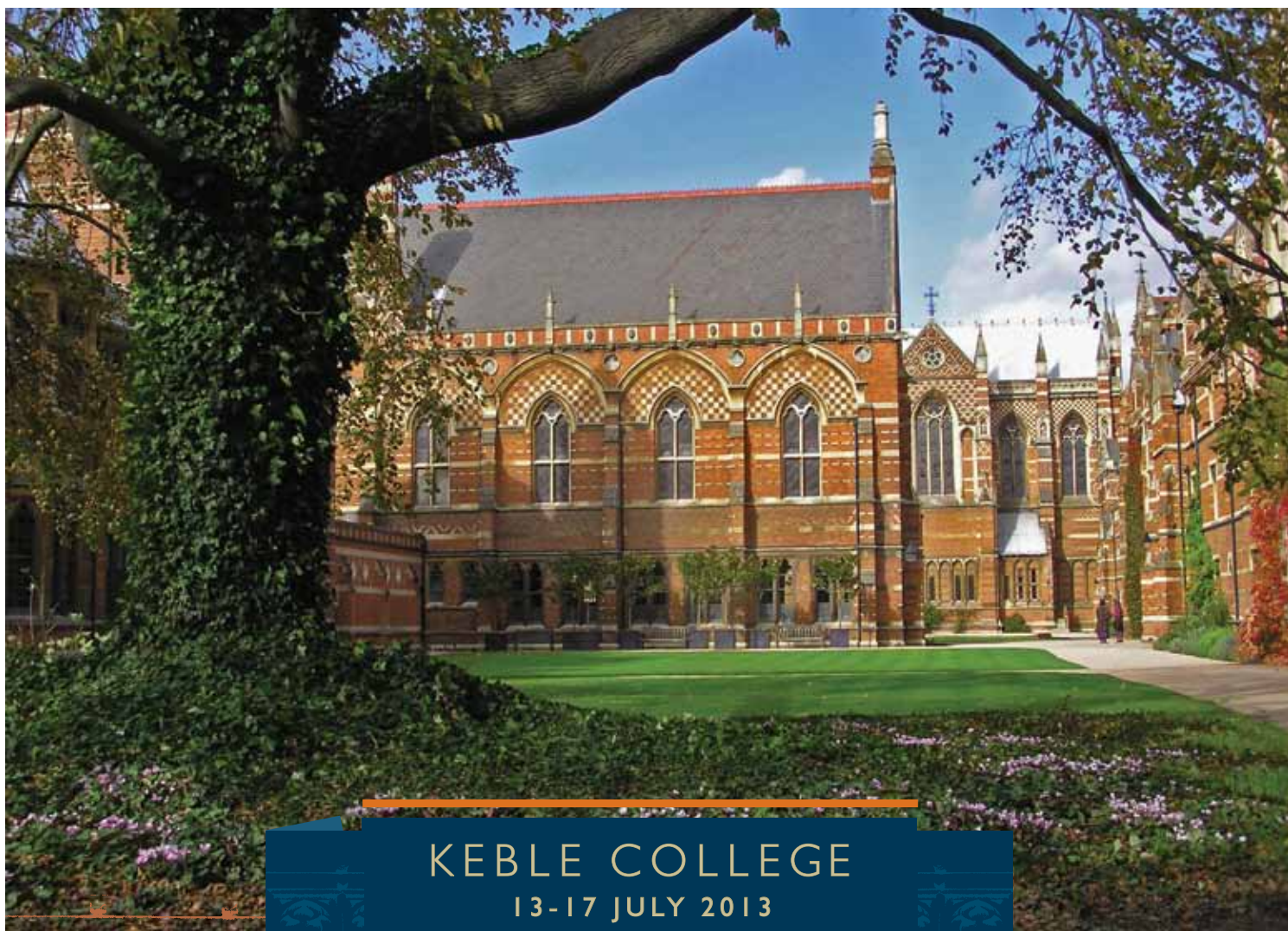


8<sup>TH</sup> BIENNIAL SYMPOSIUM OF THE  
INTERNATIONAL EOSINOPHIL SOCIETY, INC.



# FINAL PROGRAM

13-17 JULY 2013    KEBLE COLLEGE    OXFORD – UNITED KINGDOM



KEBLE COLLEGE

13-17 JULY 2013

OXFORD

UNITED KINGDOM

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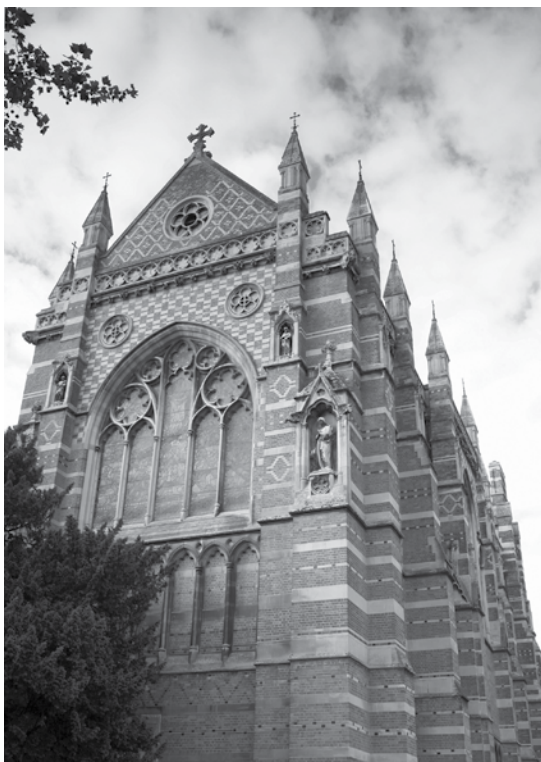
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# TABLE OF CONTENTS

Welcome.....	3
Acknowledgements .....	4
General Information.....	5
Speaker Disclosures .....	6
Scientific Program.....	7
Awards .....	18
In Memoriam .....	22
Invited Speaker Abstracts.....	23
Oral Abstracts.....	47
Poster Abstracts.....	67
Participant List .....	151

After two years of planning, the meeting has finally arrived and we would like to extend a warm welcome to all of you eosinophiles that have made the journey to Oxford for the 8th Biennial Symposium of the International Eosinophil Society, Inc. This is a particularly auspicious year for eosinophils as there are a number of anti-eosinophil treatments that are reaching an advanced state of development.

As in prior years, the meeting will be a potpourri of the latest advances in basic and clinical research, presented as state-of-the-art talks by leaders in the field, cutting edge lectures on new findings, and oral presentations and posters taken from the submitted abstracts. There will be plenty of opportunities for discussion during the three poster sessions, and prizes for the best posters and oral presentations of new work by trainees. New to this meeting is a session on eosinophil methodology designed for scientists starting out in the field who wish to learn how to interrogate eosinophil function. The final day of the meeting will be devoted to the 6th in a series of satellite clinical workshops focused on the diagnosis and treatment of hypereosinophilic syndromes.

Of course, there will be some free time in the afternoons to mingle with colleagues amidst the glories of Oxford under what we hope will be warm sunny skies, although this being England that is far from certain.

Finally, we would like to extend a special greeting and our gratitude to the meeting sponsors, without whom this meeting would not have been possible.

We hope you enjoy the meeting.



Amy Klion  
President



Andrew Wardlaw  
Scientific Program Director

# ACKNOWLEDGEMENTS

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

## SYMPOSIUM SUPPORTERS

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### Speaker Travel Support

PhD Program MOLIN at the Medical University of Graz, Austria

Roche

### Grant Support

National Institutes of Health

*Funding for this conference was made possible [in part] by 1R13AI106160-01 from the National Center for Advancing Translational Sciences and the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.*

### We thank the following individuals for their outstanding fundraising efforts on behalf of the IES.

Steven Ackerman, Andrew Wardlaw, Tim Williams

### In Kind Donation

Foley Family Wines

## Coffee Breaks

Coffee breaks are included in the registration fee for attendees and will be served daily. Coffee will be served in the Arco Room; please check the Scientific Program for exact times.

## Continuing Professional Development (CPD)

The 8th Biennial Symposium has been approved by the Federation of the Royal Colleges of Physicians of the United Kingdom for 30 category 1 (external) CPD credit(s).

## Education Sessions

All education sessions will take place in the O'Reilly Theatre located in the Sloane Robinson Building.

## Evaluations

Evaluation forms will be sent via email following the meeting.

## Meals

Breakfast, lunch and dinner will be available for those attendees who booked accommodations at Keble College or who purchased the meal plan separately. Please note that additional meals will not be available for purchase onsite. All meals will take place in the Dining Hall Building.

## Poster Sessions

All poster sessions will take place in the Douglas Price Room located in the Sloane Robinson Building. An assortment of beverage and snacks will be served. Poster presenters will stand next to their posters during their assigned session and be available for questions and discussions.

## Registration

The registration desk will be located in the Sloane Robinson Building on all days of the Symposium.

### **Hours:**

Saturday, 13 July	15:00-20:00
Sunday, 14 July	07:30-13:30 14:30-19:30
Monday, 15 July	07:30-13:00 15:00-17:30
Tuesday, 16 July	07:00-12:30 13:30-17:30
Wednesday, 17 July	08:00-12:00 13:00-14:00

## Social Events

### **Welcome Reception**

Saturday, 13 July, 18:00-19:30

The Welcome Reception will be held at Keble College outside in the Liddon Quad. If weather does not permit the event to be outside, the reception will be in the Arco Building. Refreshments and appetizers will be provided.

### **Pitt Rivers Museum Reception**

Monday, 15 July, 19:30-21:00

The Pitt Rivers Museum displays archaeological and anthropological collections of the University of Oxford. The museum is located within walking distance from the College. Refreshments and appetizers will be provided.

## Speaker Preview Room

A speaker preview room will be available for you to review and make changes to your slides. Speakers will need to bring their presentation on a USB flash drive and will upload their presentation in the session room.

# SPEAKER DISCLOSURES

The following speakers have declared no financial conflict of interest:

Rafeul Alam	Sophie Fillon	Paneez Khoury	Helene Rosenberg
Christianne Bandeira-Melo	Gerald Gleich	Fanny A. Legrand	Hans-Uwe Simon
Sarah Bettigole	Matthieu G. Groh	Kelly G. Magalhães	Christof Straub
Derek K. Chu	Grzegorz Helbig	Jun Miyata	Peter Valent
Van Trung Chu	Cory Hogaboam	Jenny Mjösberg	Catherine Vial
Nicola Diny	Elizabeth Jacobsen	Josiane S. Neves	Yui-Hsi Wang
Kimberly Dyer	Jean Emmanuel Kahn	Caroline M. Percopo	Lian Willetts
Neda Farahi	A. Barry Kay	Senbagavalli Prakash Babu	Tina W. Wong

The following speakers have disclosed a financial conflict of interest:

Steven Ackerman <i>APFED, Grant Support; NASP/FAAN Research Foundation, Grant Support</i>	Leo Koenderman <i>Dutch Asthma Foundation, Grant; Dutch CF Foundation, Grants; Dutch Organization Fun. Research, Grants</i>
Judith Appleton <i>NIH, Grant Support; USDA, Grant Support</i>	James Lee <i>Amgen, Consultant; Cephalon, Consultant; NIH, Research Support; American Heart Association, Research Support; Laboratoires d'Excellence, Proposal Review Jury Member</i>
Bruce Bochner <i>Allakos, Advisory Board, Stock; Merck, Advisory Board; Teva, Consultant; Sanofi-Aventis, Consultant; United States Diagnostic Standards, Consultant; Medicis, Consultant; Pharmacyclics, Consultant; Hoffman-LaRoche, Consultant; Tarsa Therapeutics, Consultant; Glycomimetics, Stock; Elsevier, Royalties; UpToDate, Royalties; NIH, Grant</i>	John Matthews <i>Genentech, Inc, employee</i>
Ester Boix <i>Ministerio de Educación, Spanish Government, Research Support; Generalitat de Catalunya, Research Support</i>	Andrew McKenzie <i>Janssen, licensed anti-IL-25 antibody</i>
William Busse <i>Merck, Advisory Board; Amgen, Consultant; Novartis, Consultant; GlaxoSmithKline, Consultant; MedImmune, Consultant, Genentech, Consultant; National Institutes of Health, Principal Investigator</i>	Parameswaran Nair <i>Cellometrics, Advisor; Accufilter, Patent; GlaxoSmithKline, Investigator; Novartis, Investigator; AstraZeneca, Investigator</i>
Nick Cross <i>Novartis, Honorarium, Research Support</i>	Donald Raible <i>MedImmune, employee</i>
John Fahy <i>Merck, Consulting; Regeneron, Consulting; Pathway Therapeutics, Consulting; Boehringer Ingelheim, Consulting; Cytokinetics, Consulting; University of Calgary, Honorarium; US National Institutes of Health, Research Grants</i>	Marc Rothenberg <i>Teva Pharmaceutical, Royalty; Immune Pharmaceutical, Consultant; NIH, Principal Investigator; Department of Defense, Collaborating Investigator; US-Israel Binational Foundation, Co-Principal Investigator</i>
Akos Heinemann <i>Austrian Science Funds, Research Grant; Austrian National Bank, Research Grant; Protaffin PLC, Research Grant</i>	Florence Roufosse <i>GlaxoSmithKline, Consultant</i>
Mats Johansson <i>Guidepoint Global, Consulting Fee; NIH, Co-investigator</i>	Alex Straumann <i>Falk, Study Sponsor, Consultant; Oxagen, Study Sponsor; Pfizer, Consultant</i>
Hirohito Kita <i>Novartis, Honoraria; Merck Sharp &amp; Dohme (MSD), Honoraria; GlaxoSmithKline, Honoraria; NIH, Research Grant; Mayo Foundation, Research Grant</i>	Andrew Wardlaw <i>Pfizer, research grant; GlaxoSmithKline, research grant; Teva, Honorarium</i>
Amy Klion <i>Bill and Melinda Gates Foundation, Subcontractee</i>	Peter Weller <i>GlaxoSmithKline, Consultant; NIH, principal investigator for grants</i>
	Michael Wechsler <i>GlaxoSmithKline, Research Support, Consultant; Merck, Consultant; Novartis, Consultant; Boston Scientific, Consultant; NIH, Research Support</i>
	Nives Zimmerman <i>NIH, Grant Support</i>

## SATURDAY, 13 JULY

### 16:45-17:00 Welcome

Amy Klion, United States, President  
Andrew Wardlaw, United Kingdom, Scientific Program Chair

Chair: Timothy Williams, United Kingdom

17:00-18:00

#### **The early days of the discovery of the eosinophil**

A. Barry Kay, United Kingdom

**Welcome Reception** – 18:00-19:30 (Liddon Quad)

**Dinner** – 19:30-21:00 (Dining Hall)

20:00-21:00

#### **Eosinophil Jeopardy – An eosinophil quiz**

Bruce Bochner, United States

## SUNDAY, 14 JULY

**Breakfast** – 07:00-08:00 (Dining Hall)

**Session 1: Control of eosinophil production** – 08:00-10:00

Chairs: David Broide, United States  
Stephane Esnault, United States

08:00-08:30

#### **State of the art: Mechanisms involved in Th2 related inflammatory responses**

Andrew McKenzie, United Kingdom

08:30-09:00

#### **Cutting edge: Type 2 innate lymphoid cells in pulmonary inflammation**

Hirohita Kita, United States

09:00-09:15

Abstract speaker

#### **The transcription factor XBP-1 is a critical regulator of eosinophil development**

Sarah Bettigole, United States

09:15-09:30

Abstract speaker

#### **Genetic basis of familial eosinophilia**

Senbagavalli Prakash Babu, United States

09:30-10:00

#### **Cutting edge: Human innate immune cells**

Jenny Mjösberg, Sweden

**Coffee Break** – 10:00-10:30 (Arco Room)



**SUNDAY, 14 JULY — CONTINUED**

*Session 2: The immunoregulatory role of eosinophils – 10:30-12:30*

Chairs: Patricia Bozza, Brazil  
Lisa Spencer, United States

10:30-11:00

**State of the art: Eosinophil regulation of tissue microenvironment: Roles in repair, remodeling and fibrosis**

Steven Ackerman, United States

11:00-11:30

**Cutting edge: Eosinophils as a novel cell source of prostaglandin D2: Autocrine role in allergic inflammation**

Christianne Bandeiro-Melo, Brazil

11:30-11:45

Abstract speaker

**Activated eosinophils release high mobility group box 1 protein (HMGB1), a novel immunoregulatory mediator with airway inflammatory cell-activating capabilities**

Alexander Kurosky, United States

11:45-12:00

Abstract speaker

**A role for eosinophils in promotion of B cell proliferation**

Tina Wong, United States

12:00-12:30

**Cutting edge: Biomarkers of eosinophil activation**

Mats Johansson, United States

**Lunch and Networking – 12:30-14:30 (Dining Hall)**

**Coffee Break – 14:30-15:00 (Arco Room)**

*Session 3: Eosinophils as effector cells – 15:00-17:00*

Chairs: Allison Fryer, United States  
Ariel Munitz, Israel

15:00-15:30

**State of the art: Eosinophil effector function**

Hans-Uwe Simon, Switzerland

15:30-16:00

**Cutting edge: Role of prostanoids in eosinophil recruitment**

Akos Heinemann, Austria

16:00-16:15

Abstract speaker

**EMR1: A novel therapeutic target for eosinophilic disorders**

Fanny Legrand, United States

16:15-16:30

Abstract speaker

**Eosinophil degranulation is regulated by VAMP-7-mediated exocytosis, *in vivo***

Lian Willetts, United States

16:30-17:00

**Paul Bertics Lectureship Presentation and Lecture**

William Busse, United States

**SUNDAY, 14 JULY — CONTINUED***Poster Session I - 17:00- 19:00 (Douglas Price Room)****Control of eosinophil production***

- 1 Rapamycin potently and specifically inhibits il-5+ th2 cell proliferation by a mTORC1/S6 kinase1 dependent mechanism**  
Calman Prussin
- 2 Putting brakes on eosinophil development: RhoH in health and disease**  
Christina Stoeckle
- 3 Thymic stromal lymphopoietin promotes human eosinophil-basophil lineage commitment: A key role for tumor necrosis factor-alpha**  
Claudia C.K. Hui
- 4 *In vivo* eosinophil labeling shows a circulatory lifespan of 66-84 hours**  
Tamar Tak
- 5 Differential promoter usage and regulation of the human IL-5RA gene in developing eosinophil progenitors**  
Fan Kimberly Gao
- 6 Reveiling the death pathway leading to eosinophil cytolysis**  
Susanne Irene Radonjic-Hoesli
- 7 Mechanism of Siglec-8-mediated cell death in IL-5-activated eosinophils: Role for ROS-enhanced MEK/ERK activation**  
Nives Zimmermann
- 8 "Self-recognition" via paired immunoglobulin-like receptors is fundamental for eosinophil homeostasis**  
Netali Ben Baruch- Morgenstern
- 9 Optimized protocols for eosinophil progenitor studies**  
Kaila Schollaert
- 10 Effects of hypoxia on eosinophil degranulation, apoptosis, and sensitivity to glucocorticosteroids**  
Linsey Porter
- 11 Dexramipexole decreases blood eosinophils: Results of two clinical trials in patients with amyotrophic lateral sclerosis**  
Mary Sullivan
- 12 Micrnas control transcriptional networks that regulate eosinophil differentiation**  
Ming Yang

***The immunoregulatory role of eosinophils***

- 13 Do eosinophils have a role in graft-versus-host disease?**  
Christine Wenneras
- 14 Eosinophils control the resolution of inflammation through the 12/15-lipoxygenase pathway**  
Makoto Arita
- 15 The association of eosinophils and their cationic proteins to cancer and cancer development**  
Kristin Blom
- 16 Mouse intestinal eosinophils have an antigen presenting cell phenotype and acquire luminal antigen**  
Kalmia Smith Buels
- 17 Differential eosinophil activation in chronic and acute graft versus host disease may depend on altered cytokine responses**  
Jennie Andersson
- 18 Induction of malignant plasma cell proliferation by eosinophils**  
Tina Wong
- 19 Implication of innate immune receptors in eosinophil tumoricidal activity**  
Solène Gatault
- 20 Using the allergic immune system to target cancer: activity of IGE antibodies specific for human CD20 and MUC1**  
Pearline Teo

## SUNDAY, 14 JULY — CONTINUED

*Eosinophilic GI disease*

- 47 Eosinophil phenotype in ulcerative colitis with concomitant primary sclerosing cholangitis depends on T-cell subsets in the intestine**  
Maria Lampinen
- 48 Most eosinophils are undergoing cytolysis in eosinophilic esophagitis**  
Gerald Gleich
- 49 Treatment of eosinophilic esophagitis with the CRTH2-antagonist OC000459: A novel therapeutic principle**  
Alex Straumann
- 50 The esophageal string test quantifies luminal eosinophil and chemokine biomarkers reflective of esophageal inflammation in adults with eosinophilic esophagitis**  
Steven Ackerman
- 51 regulation of blood eosinophil phenotypes by topical corticosteroids in patients with eosinophilic esophagitis**  
Christine Lingblom

*Eosinophils in host defense*

- 52 The eosinophil cationic protein, an eosinophil granule protein effective against mycobacteria**  
Ester Boix
- 53 Glycosylation modulates the antimicrobial activity of human eosinophil cationic protein native forms**  
Ester Boix
- 54 Eosinophils: An unforeseen protagonist in influenza infection**  
Amali Samarasinghe
- 55 The role of eosinophils in immunity to the trematode helminth schistosoma haematobium**  
Shona Wilson
- 56 Th-mRSVG, modified RSVG protein from RSV A2, induces protection against RSV without immunopathogenesis**  
In Su Cheon

*Teva Sponsored Symposium – 19:00-20:30*

19:00-19:05

**Welcome and Introduction**  
David Price, United Kingdom

19:05-19:25

**Uncontrolled moderate-to-severe asthma in patients with eosinophilic disease**  
David Price, United Kingdom

19:25-19:55

**Burden of moderate- to-severe asthma: The economic impact**  
Jon Campbell, United States

19:55-20:25

**Role of IL-5 in moderate-to-severe asthma, biomarkers and the evidence for anti-IL-5 therapy**  
Michael Wechsler, United States

20:25–20:30

**Conclusions and Close**  
David Price, United Kingdom

*Dinner (Teva Symposium Attendees) - 20:30-21:30 (Dining Hall)*

**MONDAY, 15 JULY****Breakfast - 07:00-08:00 (Dining Hall)***Session 4: Eosinophilic GI disease – 08:00-10:00*Chairs: Elizabeth Jacobsen, United States  
Calman Prussin, United States

08:00-08:30

**State of the art: Pathogenesis of eosinophilic esophagitis**

Marc Rothenberg, United States

08:30-09:00

**Cutting edge: Eosinophils and the gut microbiome**

Sophie Fillon, United States

09:00-09:15

Abstract speaker

**A critical role for small intestinal eosinophils in initiating food allergic Th2 immunity**

Derek Chu, Canada

09:15-09:30

Abstract speaker

**Determination of the molecular phenotype of esophageal mucosal inflammation in children with eosinophilic esophagitis using a 1-hour esophageal string test**

Steven Ackerman, United States

09:30-10:00

**State of the art: Steroids versus allergen avoidance in the management of EO**

Alex Straumann, Switzerland

**Coffee Break – 10:00-10:30 (Arco Room)***Session 5: Eosinophils in host defense – 10:30-12:30*Chairs: Paige Lacy, Canada  
Francesca Levi-Schaffer, Israel

10:30-11:00

**State of the art: Eosinophils in host defense**

Kimberley Dyer, United States

11:00-11:30

**Cutting edge: Role of eosinophils in parasitic host defense**

Judith Appleton, United States

11:30-11:45

Abstract speaker

**Purinergic receptor P2Y<sub>12</sub> functional roles in human isolated eosinophils and in the schistosomal host response**

Josiane Neves, Brazil

11:45-12:00

Abstract speaker

**Schistosomal-derived lysophosphatidylcholine triggers IL-13 and TGF  $\beta$  secretion by eosinophils through 15-lipoxygenase dependent mechanisms**

Kelly Magalhães, Brazil

12:00-12:30

**Cutting edge: Structural elements of ECP important in host defense**

Ester Boix, Spain

**Lunch and Networking – 12:30-14:45 (Dining Hall)**

## MONDAY, 15 JULY — CONTINUED

*IES Business Meeting (All are Welcome) – 14:45-15:30**Session 6: Animal models of eosinophilic lung disease – 15:30-17:00*

Chairs: Joan Cook-Mills, United States  
Eva Sturm, Austria

15:30-16:00

**Cutting edge: Pathogenesis of allergic fungal lung disease**

Cory Hogaboam, United States

16:00-16:30

**Cutting edge: Genetically manipulated mouse models of asthma**

James Lee, United States

16:30-16:45

Abstract speaker

**Novel IL-9-producing innate helper cells promote IgE-mediated experimental food allergy**

Yui-Hsi Wang, United States

16:45-17:00

Abstract speaker

**Eosinophils are essential for progression of autoimmune myocarditis to dilated cardiomyopathy**

Nicola Diny, United States

*Poster Session II – 17:00-19:00****Eosinophils as effector cells***

- 21 Development of a suspension array assay in multiplex to simultaneously measure serum levels of eosinophil granule proteins**  
Michelle Makiya
- 22 RHO and RAC are essential for secretion of eosinophil-associated RNases from human and mouse eosinophils**  
Revital Shamri
- 23 Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps**  
Dagmar Simon
- 24 Enzymatic analysis mouse eosinophil associated ribonuclease 11**  
Kelsey Yamada
- 25 IL-5 family cytokines and eotaxin polarize suspended eosinophils to form the nucleopod, a unique uropod containing the nucleus**  
Adrian Han
- 26 Clustering of IL-5 family cytokine receptors on eosinophil nucleopod and the association between eosinophil priming and polarization**  
Adrian Han
- 27 IL-33 stimulates IL-25 release by human blood eosinophils**  
Fanny Legrand
- 28 Notch signaling mediates GM-CSF-primed human eosinophil transendothelial migration through a non-canonical signaling pathway impacting ERK phosphorylation**  
Linying Liu
- 29 Adiponectin attenuates human eosinophil adhesion and chemotaxis**  
Shigeharu Ueki
- 30 Trapping capacity and stability of eosinophil extracellular DNA nets**  
Shigeharu Ueki

## MONDAY, 15 JULY — CONTINUED

- 31 Leptin activates eosinophil leukotriene C4 synthesizing machinery: Role of PI3K and endogenous RANTES**  
Tatiana Luna
- 32 The prostaglandin D2 receptors DP and CRTH2 cooperatively regulate NFAT and SRE**  
Miriam Peinhaupt
- 33 Eosinophil granule stability and viability are critically dependent on cystatin F**  
Colin Watts
- 34 Lectin affinity-based separation of variants of Eosinophil Cationic Protein (ECP)**  
Jenny Rubin
- 35 Involvement of the cannabinoid receptor 2 in the activation and chemotaxis of human eosinophils**  
Robert Frei
- 36 The effect of HMGB1 on mouse eosinophils: Viability, chemotaxis and the expression of RAGE, TLR2 and TLR4**  
Kimberly Dyer
- 37 The C-terminus of CCR3 modulates cell surface expression and functional responses to the eotaxin family of chemokines**  
James Pease
- 38 Calcitriol reduces eosinophil cytolysis and release of cytotoxic granules**  
Francis Davoine
- 39 N-glycosylation of NOX2 mediates its trafficking to the cell surface and induces ROS-dependent surface up-regulation of BLT1 during exocytotic degranulation in human eosinophils stimulated with LTB4**  
Arim Min
- 40 Characterization of the inflammasome proteins in human eosinophils**  
Renata Nesi
- 41 The role of cryptococcus neoformans capsule in modulation of eosinophils activation**  
Thaís Amanda Pinho Silva
- 42 CD48 partially mediates staphylococcus aureus induced eosinophil activation**  
Yael Minai-Fleminger
- 43 An essential role for RAB27A in eosinophil exocytosis: Implications for airway hyperresponsiveness**  
Paige Lacy
- 44 2-arachidonoyl-glycerol activates human eosinophils: involvement of IL-5, CB2 receptors, and eicosanoids**  
Nicolas Flamand
- 45 CMRF-35-like molecule 1 (CLM-1) regulates eosinophil homeostasis by suppressing cellular chemotaxis**  
Itay Moshkovits
- 46 CMRF35-like molecule 1 (CLM-1) is required for IL-33-induced eosinophil activation**  
Dana Shik

*Pitt Rivers Museum Social – 19:30-21:00 (Appetizers & Cocktails)*

## TUESDAY, 16 JULY

**Breakfast** - 07:00-08:00 (Dining Hall)*Session 7: Eosinophilic respiratory disease – 08:00-12:30*Chairs: Bruce Bochner, United States  
Michel Laviolette, Canada

08:00-08:30

**Cutting edge: Phenotyping eosinophilic asthma**

John Fahy, United States

08:30-09:00

**Cutting edge: Mepolizumab and reslizumab in the treatment of asthma**

Andrew Wardlaw, United Kingdom

09:00-09:15

Abstract speaker

**The role of type 2 innate lymphoid cells and IL33 in persistence of asthma**

Rafeul Alam, United States

09:15-09:30

Abstract speaker

**Use of 111-indium-labelled autologous eosinophils to establish *in vivo* kinetics of human eosinophils**

Neda Farahi, United Kingdom

09:30-10:00

**Cutting edge: Benralizumab in the treatment of eosinophilic lung disease**

Donald Raible, United States

**Coffee Break** – 10:00-10:30 (Arco Room)Chairs: Rafeul Alam, United States  
John Fahy, United States

10:30-10:45

Abstract speaker

**Activated eosinophils protect against a lethal respiratory virus infection**

Caroline Percopo, United States

10:45-11:00

Abstract speaker

**Adoptive transfer of activated pulmonary eosinophils is sufficient to restore Th2 pulmonary inflammation in eosinophil-deficient PHIL mice in a model of acute asthma**

Elizabeth Jacobsen, United States

11:00-11:30

**Cutting edge: Identification of the eosinophil phenotype using point-of-care biomarkers**

Parameswaran Nair, Canada

11:30-11:45

Abstract speaker

**Impaired P2X1 receptor function in eosinophils from asthmatic patients**

Catherine Vial, United Kingdom

11:45-12:00

Abstract speaker

**Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma**

Jun Miyata, Japan

12:00-12:30

**Cutting edge: Lebrikizumab in the treatment of asthma**

John Matthews, United States

**Lunch and Networking** – 12:30-13:30 (Dining Hall)

**TUESDAY, 16 JULY — CONTINUED***Session 8: Eosinophil laboratory techniques – 13:30-15:00*

Chair: Simon Hogan, United States

13:30-15:00

**Human and mouse eosinophils: Isolation, generation and related applications**

Helene Rosenberg, United States

**It's Christmas in July: The availability and utility of eosinophil-specific reagents and mouse models from Lee Laboratories**

James Lee, United States

**Methods for translational eosinophil research: From the bench to bedside and back**

Leo Koenderman, The Netherlands

**Flow cytometry of eosinophils: From identification to functional assays**

Nives Zimmerman, United States

*Coffee Break – 15:00-15:30 (Acro Room)**Session 9: IES award lectures – 15:30-17:00*

Chair: Amy Klion, United States

15:30-16:00

**Gleich Award Presentation and Lecture**

Van Trung Chu, Germany

16:00-17:00

**Ehrlich Award Presentation and Lecture**

Peter Weller, United States

*Poster Session III - 17:00-19:00***Animal models of eosinophilic lung disease**

- 57 Eosinophils influence the inflammatory phenotype of asthma in an inducible knock-in eosinophil-deficient mouse model of asthma**  
Elizabeth Jacobsen
- 58 Impaired airway eosinophil migration into the paratracheal lymph nodes in LTC<sub>4</sub> synthase-deficient mice**  
Haibin Wang
- 59 A mouse with efficient expression of CRE-recombinase exclusively in eosinophils**  
Alfred Doyle
- 60 Mice deficient in the alpha 2,3 sialyltransferase ST3gal-III selectively manifest enhanced allergic eosinophilic airway inflammation**  
Takumi Kiwamoto
- 61 A novel method to explore eosinophil *in vivo* trafficking in the mouse**  
Eva Sturm
- 62 Maternal alpha-tocopherol supplementation of allergic female mice inhibits allergic inflammation in offspring**  
Joan Cook-Mills
- 63 Ozone induced airway hyperreactivity is exacerbated by blockade of eosinophil recruitment into lungs**  
Sarah Wicher
- 64 Mouse models to study mechanisms of eosinophil secretion during inflammatory responses**  
Kennedy Bonjour
- 65 Directed delivery of antigen to respiratory mucosal surfaces; A mouse model to address local and remote mucosal effects in allergic airway inflammation**  
Maytal Bivas-Benita
- 66 Prevention of inflammatory colitis by a recombinant enzyme from the schistosome helminth parasite: Induction of A Th2 response with eosinophils and alternatively activated macrophages**  
Virginie Driss



## TUESDAY, 16 JULY — CONTINUED

*Eosinophilic respiratory disease*

- 67 The clinical profile of UK asthma patients with raised blood eosinophils**  
David Price
- 68 Aspirin-induced asthma sub-phenotypes identified by latent class analysis**  
Grazyna Bochenek
- 69 Identification of genes expressed by human airway eosinophils after an *in vivo* allergen challenge**  
Stephane Esnault
- 70 Semaphorin 7A is expressed on human airway eosinophils after segmental allergen challenge *in vivo* and is upregulated by the common  $\beta$  chain cytokines *in vitro***  
Stephane Esnault
- 71 Whole body counting to quantify the distribution and fate of Indium-111-labelled eosinophils in healthy and asthmatic volunteers**  
Chrystalla Loutsios
- 72 Eosinophil and nasal allergen challenge**  
Trevor Hansel
- 73 Role of CCL26 in the recruitment of human eosinophils: A putative role in asthma severity**  
Nicolas Flamand
- 74 During inflammation refractory eosinophils stay in peripheral blood while activated counterparts dwell in tissue**  
Bart Hilvering

*Hypereosinophilic disease*

- 75 PEG-interferon treatment of eosinophilic otitis media-report of 2 cases**  
Joseph Butterfield
- 76 Gleich's syndrome of the gut: A new disorder or an under-recognized phenomenon?**  
Paneez Khoury
- 77 Hematologic malignancies in patients with hypereosinophilia and hypereosinophilic syndrome**  
Catherine Weiler
- 78 Evaluation of effects of PDGFR-blocking tyrosine kinase inhibitors on growth and migration of neoplastic eosinophils in FIP1L1/PDGFR $\alpha$  Chronic Eosinophilic Leukemia (CEL)**  
Irina Sadovnik
- 79 The WHO classification of mastocytic diseases does not incorporate disease such as ETV6-ABL1: A distinct genetic group**  
Finella Brito-Babapulle
- 80 Clozapine induced eosinophilia with perimyocarditis**  
Urs Steiner
- 81 Is peripheral blood eosinophilia being investigated appropriately? A retrospective review of 89 patients**  
Melanie York
- 82 Causes of an eosinophilia presenting to a specialist hypereosinophilic clinic**  
Melanie York
- 83 Unexpected cytokine profiles suggest differential roles of eosinophilopoietin-producing T cells in peripheral blood eosinophilia and hypereosinophilia**  
Christina Stoeckle
- 84 Invasive cutaneous trichophyton rubrum infection associated with blood and tissue eosinophilia in the setting of a CARD9 deficiency**  
Florence Roufosse

*Dinner – 19:30-21:00 (Dining Hall)*

**WEDNESDAY, 17 JULY***Satellite Clinical Workshop***Breakfast** – 07:00-08:15 (Dining Hall)*Session 10: Hypereosinophilic disease – 08:15-12:00*Chairs: Jean Emmanuel Kahn, France  
Princess Ogbogu, United States

08:15-08:45

**State of the art: Diagnostic issues in HES; The example of CSS**

Michael Wechsler, United States

08:45-09:00

Abstract speaker

**Orthotopic heart transplantation in patients with eosinophil granulomatosis with polyangiitis:****Report on nine cases**

Matthieu Groh, France

09:00-09:30

**State of the art: End organ manifestations: Are they useful for differential diagnosis**

Gerald Gleich, United States

09:30-10:00

**Cutting edge: HES as a multidisciplinary disorder**

Grzegorz Helbig, Poland

**Coffee Break** – 10:00-10:30 (Arco Room)Chairs: Florence Roufousse, Belgium  
Andrew Wardlaw, United Kingdom

10:30-12:00

**Grand round: Illustrative case histories in HES**

Florence Roufousse, Belgium

Andrew Wardlaw, United Kingdom

**Lunch** (additional fee) - 12:00-13:30*First official meeting of the Clinical Subgroup of the IES**Session 11: Myeloproliferative diseases involving eosinophils – 13:30-15:30*

Chair: Joseph Butterfield, United States

13:30-14:00

**Establishment of an international network of investigators of eosinophil associated myeloproliferative disorders**

Peter Valent, Austria

14:00-14:15

Abstract Speaker

**Predictors of responsiveness to imatinib in hypereosinophilic syndrome**

Paneez Khoury, United States

14:15-14:45

**Clinical manifestations and biomarkers of eosinophil-associated myeloproliferative disease**

Jean Emmanuel Kahn, France

14:45-15:15

**What do genetic mutations tell us about the pathogenesis and treatment of eosinophilic associated myeloproliferative disease**

Nick Cross, United Kingdom

15:15-15:30

**Wrap-up**

# PAUL BERTICS LECTURESHIP

Paul was the Robert Turell Professor of Physiology (endowed chair), Kellett Professor of Biomolecular Chemistry at the University of Wisconsin (UW). Paul was extremely dedicated to the students at UW serving as chair of Medical School admissions for years, instructing over 3,000 medical students, training over 55 graduate and postdoctoral students and mentoring countless undergraduates. He received numerous awards, including the student-selected UW Medical School teaching award twice and the UW Distinguished Teaching Award - Chancellor's Teaching Award. Paul was an editor of the Journal of Immunology and the Chief Science Advisor of Platypus Technologies, Fitchburg.

Dr. Paul Bertics was a distinguished scientist focused on understanding molecular and biochemical mechanisms of inflammatory cells, especially eosinophils. He was an active member of the International Eosinophil Society and attended all meetings. We have established this award to remember Paul's contribution to our field not only for his scientific excellence, but also because of his love of teaching and mentoring students.

It is an honor to present the first Paul Bertics Award to Dr. William W. Busse who was a personal friend, colleague and mentor of Dr. Bertics. Dr. Busse was also a graduate of UW and its medical school. Following an internship at Cincinnati General Hospital, he completed a residency in Internal Medicine and fellowship in Allergy and Immunology at UW, and has been on its faculty since 1974. He has served as head of the Allergy and Clinical Immunology Section (1978-2004) and George R. and Elaine Love Professor and Chair of Medicine (2005-2009).

Dr. Busse's research interests have focused on the mechanism of asthma with particular interests in eosinophilic inflammation and rhinovirus-induced asthma for which he has had long-standing NIH support. He is also the Principal Investigator on an NIH-NIAID Inner City Asthma Consortium, which is funded to study immune-based therapy for asthma in inner-city children.

Dr. Busse has been the director on the American Board of Allergy and Immunology (1989-1995). He was on the Advisory Council, National Institutes of Health, National Heart, Lung and Blood Institute (1996-2000). Dr. Busse was a member of the Expert Panel on the Guidelines for the Diagnosis and Management of Asthma (1989, 2002), and is presently chair of this group, and a member of the Board of External Experts, National Heart, Lung and Blood Institute (2006-present). From 1994-2004, he was on the AAAAI Board of Directors and served as President of the AAAAI in 2000-2001.

Dr. Busse's awards include election into the Association of American Physicians, the Folkert Belzer Life Achievement Award, the American Thoracic Society Award for Scientific Accomplishments, and the Citation Award for Achievement from UW.

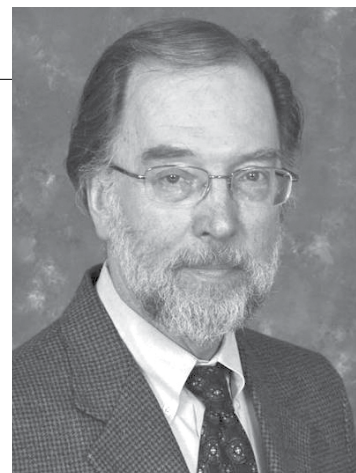
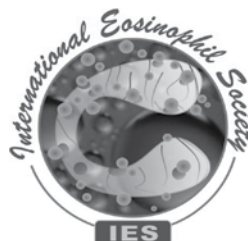
## Recipient of the 2013 Bertics Lectureship



**THE PAUL BERTICS LECTURESHIP  
INTERNATIONAL EOSINOPHIL  
SOCIETY, INC.  
2013**

*William W. Busse, MD*

*Paul was a distinguished eosinophil researcher and an active member of the IES. He was a dear friend and colleague to many of the members of our society.*



Dr. Van Trung Chu will receive the second Gerald Gleich prize to be awarded at the 8th Biennial Symposium of the International Eosinophil Society, Inc. in Oxford, UK. The prize was specifically created to recognize individuals who have published high impact findings during the intervals since the preceding meeting. This award was named in honor of our esteemed colleague, Dr. Gerald Gleich, whose career has been devoted to the exploration of the eosinophilic leukocyte and to the elucidation of its role in health and disease. The prize is bestowed by a consulting committee and Dr. Gerald Gleich.

Dr. Van Trung Chu was selected on the basis of his first-authored publication "Eosinophils are required for the maintenance of plasma cells in the bone marrow", *Nat Immunol.* 2011 12(2):151-159. doi: 10.1038/ni.1981. The work was carried out in the laboratory of Dr. Claudia Berek of the Deutsches Rheuma-Forschungszentrum, Institut der Leibniz-Gemeinschaft, Berlin, Germany. In this work, Dr. Chu and Dr. Berek showed that in the bone marrow, eosinophils are required for the maintenance of long-lived plasma cells. Therefore, eosinophil-directed therapy might provide a new therapeutic approach for the treatment of autoimmune diseases and other diseases in which plasma cells contribute to the pathogenesis. Dr. Chu's research currently focuses on eosinophil-plasma cell interaction. We are pleased that Dr. Chu will be joining us at the meeting to receive this award.

### Recipient of the 2013 Gleich Award



**THE GERALD J. GLEICH AWARD  
INTERNATIONAL EOSINOPHIL  
SOCIETY, INC.  
2013**

*Van Trung Chu, PhD*

*In recognition of the most intriguing,  
high impact finding related to  
eosinophil biology published in the  
years 2011-2013.*



# EHRlich LECTURESHIP

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

Peter F. Weller, M.D. is Chief of the Divisions of Infectious Diseases and Allergy and Inflammation and Vice-Chair for Research in the Department of Medicine at Beth Israel Deaconess Medical Center. He is a Professor of Medicine at Harvard Medical School, and a Professor of Immunology and Infectious Diseases at the Harvard School of Public Health. Dr. Weller received his M.D. degree from Harvard Medical School and completed his medical training at the Peter Bent Brigham Hospital, his infectious diseases training at the Laboratory of Parasitic Diseases, National Institutes of Health and at Massachusetts General Hospital and his allergy training at the Brigham and Womens' Hospital. He is board-certified in internal medicine, infectious diseases and allergy and immunology. Dr. Weller's research studies have focused on delineating basic mechanisms of leukocyte functioning in forms of inflammation. The two principal areas of investigation are: 1) the immunobiology of eosinophilic leukocytes, and 2) the intracellular regulation and compartmentalization of inducible mediators of inflammation in neutrophils and other leukocytes. These investigations are pertinent to the roles of eosinophils in allergic and anti-parasite immune responses and to the cellular biology of leukocytes underlying their functions in infectious and immune inflammatory responses.

## Recipient of the 2013 Ehrlich Lectureship



**PAUL EHRlich LECTURESHIP  
INTERNATIONAL EOSINOPHIL  
SOCIETY, INC.  
2013**

*Peter F. Weller, MD*

*For seminal work in the field of  
eosinophil immunobiology and  
physiology and outstanding  
contributions to the understanding of  
the role of eosinophils in  
health and disease.*



**2013 International Eosinophil Society Service Award to Redwan Moqbel, PhD**

The International Eosinophil Society, Inc., its leaders and members present to Professor Redwan Moqbel the distinguished Service Award both for his cardinal leadership in helping found and advance the International Eosinophil Society, Inc. and for his career-long contributions to innovative studies of the immunobiology of eosinophils. Redwan's early research focused on helminth immunity and evolved with a longstanding focus on eosinophils to embrace novel studies of eosinophils pertinent to allergic inflammation. Redwan's professional career developed early in London and thereafter was noted by his professorial and leadership roles in both Alberta and Manitoba, Canada. Throughout his career, eosinophil immunobiology remained central to his interests and research; he contributed substantially to advancing our understanding of varied aspects of eosinophil functioning and of mentoring trainees.

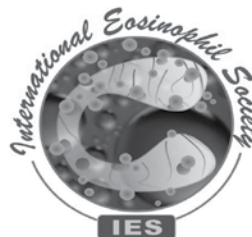
In the evolution of the International Eosinophil Society, Inc., Redwan was a central contributor. Following an eosinophil meeting in Lund, Sweden in 1999, there was a consensus amongst eosinophil immunobiologists that a more formal Society needed to be created. To this end, Redwan stepped up and organized the first International Eosinophil Society, Inc. meeting in Banff, Alberta in 2001. Redwan served as Secretary of the nascent International Eosinophil Society, Inc. from 2001-2003 and then as the Society's President from 2003-2005. During his leadership the Society progressively developed and mounted successful biennial meetings. Redwan was also critically involved in helping formalize the International Eosinophil Society, Inc. with adoption of a requisite Constitution and Bylaws and more recently professional management. In addition to what is evident in Redwan's official roles in the International Eosinophil Society, Inc. we also recognize his many behind the scenes contributions to benefit the International Eosinophil Society, Inc. from surviving published challenges to roles of eosinophils in asthma (and consequent loss of funding support) to his sagacious and devoted advocacy to assuring that the global community of eosinophil immunobiologists would have a societal home in the International Eosinophil Society, Inc.



**SERVICE AWARD  
INTERNATIONAL EOSINOPHIL SOCIETY, INC.  
2013**

*Redwan Moqbel, PhD*

*In recognition of dedicated service  
to the International Eosinophil Society, Inc.  
and to the larger community of  
eosinophil scientists*



## **Paul Bertics, PhD**

Paul was a distinguished eosinophil researcher, and an active member of the IES and a regular attendee at our biennial meetings. He was a dear friend and colleague to many of the members of our society. A memorial lectureship at the biennial IES meeting is being planned and contributions to this are being accepted. Paul was a 1974 graduate of Carlsbad High School, Carlsbad, Calif., and received his Magna cum laude B.S. with highest honors in biochemistry from the University of California, Los Angeles in 1978, his PhD in physiological chemistry in 1984 from the University of Wisconsin-Madison, and his postdoctoral training at the University of California, San Diego from 1984-86. Paul joined the UW faculty in 1986 and held the Robert Turell Professor of Physiology (endowed chair), Kellett Professor of Biomolecular Chemistry, a member of the Executive Committee of the UW Comprehensive Cancer Center, and a Co-Director in the Material Sciences Research Center in the UW School of Engineering. Paul was extremely dedicated to the students at the UW serving as chair of Medical School admissions for years, instructing over 3,000 medical students, training over 55 graduate and postdoctoral students and mentoring countless undergraduates.

## **Frederick (Freddy) Hargreave, MD, MB, FRCPC, FRCP**

He was born in Hong Kong and completed his medical school training at the University of Leeds (Leeds, United Kingdom). After completing his initial clinical training, Freddy moved to London in 1964 to work as a house officer in respiratory medicine with Dr. E.J. Moran Campbell at the Hammersmith Hospital. Freddy Hargreave joined the Department of Medicine at McMaster University (Hamilton, Ontario) in 1969, and was based at the Firestone Institute for Respiratory Health (Hamilton), where he spent his entire career. Within 10 years, the studies led by Freddy Hargreave had changed the way that asthma was diagnosed, and had paved the way to future studies that have revolutionized its treatment. In particular, Dr. Don Cockcroft (who was Freddy's first clinical fellow) described the methodology for the measurement of airway hyper-responsiveness in asthma. Together, they demonstrated that this was a crucial component of the disease and present in all patients who had current symptoms. They also showed that the degree of airway hyper-responsiveness was related to the amount of treatment needed to manage asthma. The article describing this methodology is a citation classic, having been cited almost 1,800 times.

**THE EARLY DAYS OF THE DISCOVERY OF THE EOSINOPHIL****A. Barry Kay**

In 1879, Paul Ehrlich (1854-1915) at the age of 25 published his technique for staining blood films and his method for differential blood cell counting using coal tar dyes, although curiously the first published work on the eosinophil ("Contribution to Knowledge of Granulated Connective Tissue Cells and of Eosinophil Leukocytes") did not mention the cell at all. The article was based on Paul Ehrlich's presentation to the Physiological Society of Berlin on January 17, 1879 and so it is assumed that the section on the eosinophil had been deleted. It remains a mystery as to what Ehrlich actually said about the cell in his lecture. It is in his next paper ("About the Specific Granulations of the Blood"), also based on a presentation to the Physiological Society of Berlin in 1879, that the eosinophil is mentioned for the first time. When discussing granule types in the white cells of vertebrates he writes; "The most important of these granulations by far is the eosinophil or alpha granulation, about which I have already spoken before this society on the 17<sup>th</sup> January of this year. These alpha granules are characterised by their affinity for a wide range of acid coal tar dyes."

Not only did Ehrlich discover the eosinophil, he also described many of its features in detail and speculated, for the most part correctly, on its formation and function. He observed that the alpha granules were either round, or had the shape of short rods with rounded ends, that there was variation in the number of nuclear lobes and that the number of granules fluctuates from cell to cell. Ehrlich observed eosinophils in all species of animals he studied and that there were large numbers in the bone marrow leading to the suggestion that this was the site of their formation. Moreover he noticed a second type of granule in these bone-marrow-derived eosinophils which stained black with an eosin-indulin-glycerin stain. He called these "beta granules" and thought they probably represented a developmental stage in the formation of alpha granules.

Although these achievements were remarkable others had almost certainly observed the cell before Paul Ehrlich. In 1846 the British physiologist and ophthalmologist Thomas Wharton Jones (1808-1891) described the "granule blood-cell" in large number of blood cells including the lamprey, frog, fowl, horse, elephant and man. He "borrowed" the term granule cell from the eminent pathologist Julius Vogel (1814-1880) who had used the term to describe cells he had observed in certain inflammatory exudates. Vogel in turn deferred to the Belgian physician Gottlieb (Théophile) Gluge (1812-1898) who had described "compound inflammatory globules" which he had sometimes found in pus or alone in serum. The drawings of Jones, Gluge and Vogel all show cells with a remarkable resemblance to what we now know as the eosinophil.

During this presentation I will discuss aspects of the life and times of these early pioneers as well the various views, current at the time, as to the function of the eosinophil.



## **MECHANISMS INVOLVED IN TH2 RELATED INFLAMMATORY RESPONSES**

Andrew N.J. McKenzie

MRC Laboratory of Molecular Biology, Cambridge, UK.

Innate lymphoid cells (ILC) are newly identified members of the lymphoid lineage with emerging roles in mediating immune responses, and regulating tissue homeostasis and inflammation. We have demonstrated that ILC2, in addition to the classical Th2 cells, produce the type-2 cytokines IL-4, IL-5, IL-9, and IL-13, and play critical roles in the initiation of the type-2 response. Notably, ILC subsets appear to be particularly prevalent at mucosal surfaces, which are constantly exposed to infectious agents in the external environment. The fact that ILC2 rapidly secrete high levels of IL-5 and IL-13, resulting in eosinophilia and mucus hypersecretion, suggests that these cells have evolved to provide a potent innate effector response to combat intestinal parasitic helminth infections. ILC2 require the transcription factors GATA3 and RORa for their development from lymphoid precursors. Ongoing studies using new reagents, including ILC2-deficient mice, are aimed at dissecting the roles of ILC2 in infection, asthma and allergy, and their interactions with innate and adaptive immune responses.

**Grant support:** *Supported by grants from MRC, the American Asthma Foundation, Janssen*

**ROLES OF IL-33-RESPONSIVE INNATE LYMPHOID CELLS IN EOSINOPHILIC AIRWAY INFLAMMATION AND ASTHMA**

Hirohito Kita

Division of Allergic Diseases, Departments of Internal Medicine and Immunology, Mayo Clinic, Rochester, MN 55905, U.S.A.

Innate immunity provides the first line of response to invading pathogens and a variety of environmental insults. Recent studies identified novel subsets of innate lymphoid cells that are capable of mediating immune responses in mucosal organs. Here we describe a subset of lymphoid cells, namely type 2 innate lymphoid cells (ILC2s), that is involved in innate type-2 immunity in the lungs. Airway exposure of naïve BALB/c or C57BL mice to IL-33 results in a rapid (< 12 h) production of IL-5 and IL-13 and marked airway eosinophilia independently of adaptive immunity. In the lungs of non-sensitized naïve mice, IL-33-responsive cells were identified that have a lymphoid morphology, lack lineage markers, highly express CD25, CD44, Thy1.2, ICOS, Sca-1 and IL-7Ra, and require IL-7Ra for their development, consistent with immunological characteristics of ILC2s. Airway exposure of naïve mice to a clinically relevant ubiquitous fungal allergen, *Alternaria alternata*, increases bronchoalveolar lavage levels of IL-33, followed by IL-5 and IL-13 production and airway eosinophilia without T or B cells. This innate type-2 response to the allergen is nearly abolished in mice deficient in IL-33 receptor (i.e. ST2), and ILC2s in the lungs are required and sufficient to mediate the response.

Subsequent analyses showed that mouse lung ILC2s enhance proliferation of naïve CD4<sup>+</sup> T cells and promote production of Th2 cytokines including IL-4, IL-5 and IL-13 *in vitro*. ILC2s regulate T cell function by direct contact through OX40/OX40L interaction. We further revealed that lung ILC2s promote IgA, IgE, IgG1 and IgM antibody production by both B1 and B2 cells, and that this stimulatory effect of ILC2s is mediated at least in part by ILC2 production of cytokines IL-5.

We also found human peripheral blood contain a human counterpart of mouse ILC2s. Human peripheral blood mononuclear cells (PBMCs) respond to IL-33 or IL-25 by producing IL-5 and IL-13. CD3<sup>+</sup> and CD16<sup>+</sup> cells are not required for the response. IL-5 and IL-13 production is induced by IL-33 or IL-25 without antigens in PBMCs from normal individuals as well as from patients with allergic rhinitis or allergic asthma. Importantly, PBMCs from asthma patients produce significantly higher amounts of IL-5 and IL-13 as compared to those from normal individuals and patients with allergic rhinitis. Finally, when cultured with IL-33, Lin<sup>-</sup>CD127<sup>+</sup>CRTH2<sup>+</sup> cells isolated from PBMCs by FACS sorting produce a large quantity of IL-5 and IL-13, but not IL-4, IL-17 or IFN- $\gamma$ .

In conclusion, mouse and human ILC2s respond to IL-33 and vigorously produce Th2-type cytokines, resulting in robust eosinophilic inflammation even in the absence of T cells or B cells. Furthermore, ILC2s promote both T cell- and B cell-mediated immune responses. Thus, ILC2s may provide a novel mechanism for type-2 immunity and induction of eosinophilic airway diseases such as asthma.

**Grant support:** Supported by NIH grant R01 AI34486 and by Mayo Foundation

## **HUMAN INNATE IMMUNE CELLS**

Jenny Mjösberg

Center for Infectious Medicine, Karolinska Institute, Stockholm, Sweden

Innate lymphoid cells (ILC) is the collective term for a group of related innate lymphocytes, including NK cells and the more recently appreciated non-NK ILCs, which all lack rearranged antigen-receptors such as those expressed by T and B cells. Similar to NK cells, the newly discovered ILC depend on the transcription factor Id2 and the common  $\gamma$ -chain of the IL-2 receptor for development. However, in contrast to NK cells, non-NK ILC also require IL-7. In addition to the cytotoxic functions of NK cells, assuring protection against tumor development and viruses, all ILC contribute to a wide range of homeostatic and pathophysiological conditions in various organs via specialized cytokine-production capabilities. Recently it was proposed that, in analogy with the T helper cell family nomenclature, NK cells and IFN- $\gamma$  producing ILC would be designated ILC1. The term ILC2 should be used for ROR $\alpha$ /GATA3-dependent ILC producing IL-5/IL-13 whereas ILC3 annotates ROR $\gamma$ t-dependent ILC producing IL-22 and/or IL-17. Here I summarize the current knowledge on ILC with a particular focus on the discovery of the human ILC1 and ILC2 populations and their roles in mucosal homeostasis and inflammation in various organs.

**PGD<sub>2</sub> AND EOSINOPHILS: MUCH MORE THAN A SIMPLE CHEMOATTRACTANT/CELL RELATIONSHIP**Christianne Bandeira-Melo

Laboratório Inflamação, Instituto de Biofísica Carlos Chagas Filho, UFRJ, RJ, Brasil.

Prostaglandin (PG) D<sub>2</sub> is a cyclooxygenase product synthesized mostly by mast cells, which constitutively express high levels of the terminal enzyme involved in PGD<sub>2</sub> synthesis, the hematopoietic PGD synthase (h-PGDS). Acting throughout two distinct receptors, named DP<sub>1</sub> and DP<sub>2</sub>, PGD<sub>2</sub> has emerged as a key mediator of allergic inflammatory pathologies. Remarkably, among PGD<sub>2</sub>-driven allergic properties, PGD<sub>2</sub> has potent chemotactic activity towards allergy-relevant effector cells, notably eosinophils and Th2 cells. Our studies investigate additional associations between PGD<sub>2</sub> and eosinophils beyond the well-characterized PGD<sub>2</sub> role as eosinophilotactic agent. Specifically, two other possible PGD<sub>2</sub>/eosinophil functional axes were addressed: (i) PGD<sub>2</sub> capability to trigger activation of eosinophil effector functions; and (ii) eosinophils as alternative cell sources of biologically active PGD<sub>2</sub>.

Our first body of data uncovered that indeed, besides eosinophil migration, PGD<sub>2</sub> displays the ability of eliciting eosinophil LTC<sub>4</sub> synthesis, a hallmark function of activated eosinophils, both *in vitro* as well as in sites of allergic inflammation. PGD<sub>2</sub>-elicited mechanism depends on an unexpected but mandatory cooperation between DP<sub>1</sub> and DP<sub>2</sub> receptors, involving DP<sub>1</sub>-driven lipid body biogenesis and LTC<sub>4</sub> synthesis compartmentalization and DP<sub>2</sub>-induced calcium influx. We also unveiled that, besides being able to control eosinophilic effector functions (such as LTC<sub>4</sub> synthesis), PGD<sub>2</sub> can also trigger eosinophil immune-modulatory functions, such as selective mobilization and rapid secretion of eosinophil granule-stored cytokines. Such phenomenon is triggered by either additive, synergistic, or even antagonistic activation of DP<sub>1</sub> and DP<sub>2</sub> receptors depending on specific cytokine selected from eosinophil granule to subsequent extracellular release. Yet, other group of interesting data on PGD<sub>2</sub>-elicited eosinophil activation demonstrates that schistosomal lipids trigger eosinophil functions (such as lipid body biogenesis, synthesis of LTC<sub>4</sub> and cytokine secretion) by a mechanism dependent on activation of DP<sub>1</sub> and DP<sub>2</sub> by schistosomal-derived PGD<sub>2</sub>.

Moving from PGD<sub>2</sub> effects on eosinophils to eosinophils as cell source of biologically active PGD<sub>2</sub>, we have shown that eosinophils indeed, like mast cells, are also capable to synthesize and, therefore, supply biologically active PGD<sub>2</sub> when properly stimulated. Constitutive expression of H-PGDS was found within non-stimulated human circulating eosinophils of healthy donors. Acute stimulation of eosinophils with arachidonic acid, human eotaxin and a varied of other non-obvious eosinophil stimuli (bradykinin, MIF and leptin) evoked PGD<sub>2</sub> synthesis, which was located at the eosinophil lipid bodies and was inhibited by pre-treatment with HQL-79, a specific inhibitor of H-PGDS. Of note, by acting on PGD<sub>2</sub> specific receptors expressed on eosinophil surface (DP<sub>1</sub> and DP<sub>2</sub>), eosinophil-derived PGD<sub>2</sub> displayed an autocrine/paracrine ability to activate cell shape-change, lipid body biogenesis and LTC<sub>4</sub> synthesis. *In vivo*, infiltrating eosinophils found at the inflammatory site of the allergic reaction were identified as a PGD<sub>2</sub>-synthesizing cell population.

Collectively, our growing body of data place eosinophils as key players able to synthesize and secrete PGD<sub>2</sub>. So, eosinophils represent an extra cell source of PGD<sub>2</sub> during allergic inflammation, which functions as an autocrine signal for activation of both effector and immune-modulatory functions of eosinophils themselves.

**Grant support:** CAPES, CNPq and FAPERJ.

**BIOMARKERS OF EOSINOPHIL ACTIVATION: INTEGRIN ACTIVATION STATES AND EOSINOPHIL RECRUITMENT IN ASTHMA**

Mats W. Johansson<sup>1</sup> and Deane F. Mosher<sup>1,2</sup>

Departments of <sup>1</sup>Biomolecular Chemistry and <sup>2</sup>Medicine, University of Wisconsin, Madison, WI, USA

**Background:** Eosinophil arrest and recruitment to the airway in asthma are mediated, at least in part, by integrins. Whether a given integrin-ligand pair mediates cell adhesion and migration depends on the activation state of the integrin. Integrins exist in an inactive bent, an intermediate-activity extended closed, and a high-activity extended open conformation. Integrin activation states can be monitored by conformation-specific monoclonal antibodies (mAbs). Studies in mice indicate that both  $\beta 1$  and  $\beta 2$  integrins mediate eosinophil recruitment to the lung.

**Methods:** Activation states of integrins on blood and airway eosinophils were monitored in several patient studies by processing unfractionated blood or BAL cells for flow cytometry and analyzing eosinophils gated based on CD14 and CD16 staining and scatter. Blood samples were from subjects with non-severe or severe asthma or from healthy controls. BAL cells were from subjects with mild allergic asthma 48 h after segmental lung antigen challenge. The *in vivo* studies were complemented by studies on adhesion and migration of purified eosinophils *in vitro*.

**Results:** The *in vitro* studies indicate that  $\alpha 4\beta 1$  and  $\alpha M\beta 2$  are the principal integrins mediating eosinophil adhesion, including to vascular cell adhesion molecule-1 (VCAM-1) and the novel  $\alpha M\beta 2$  ligand periostin. The patient studies, including an inhaled corticosteroid (ICS) withdrawal study, a segmental antigen challenge study, a virus-induced exacerbation study, a severe asthma study, and a mepolizumab study, demonstrate the following: *In vivo*, blood eosinophils have intermediate-activity  $\beta 1$  integrins, as judged by mAb N29, apparently resulting from eosinophil binding of P-selectin on the surface of activated platelets, and have a proportion of their  $\beta 2$  integrins in the intermediate conformation, as judged by mAb KIM-127, apparently due to exposure to low concentrations of circulating IL-5. Airway eosinophils have high-activity  $\beta 1$  integrins and high-activity  $\alpha M\beta 2$  that does not require IL-5. Information on how the activation states of eosinophil  $\beta 1$  and  $\beta 2$  integrins correlate with measurements of eosinophil recruitment and pulmonary function in asthma will be reviewed. To summarize, blood eosinophil N29 reactivity is associated with decreased lung function under various circumstances in non-severe asthma and KIM-127 with BAL eosinophil numbers.

**Conclusions:** Taken together, the results indicate that intermediate-activity  $\alpha 4\beta 1$  and  $\alpha M\beta 2$  of blood eosinophils are important for eosinophil arrest and consequently for recruitment and aspects of asthma. Eosinophil  $\beta 1$  integrin activation may be a biomarker in non-severe asthma.

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**PROSTAGLANDINS IN THE REGULATION OF EOSINOPHIL FUNCTION**Akos Heinemann

Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

Prostaglandins (PG) are derived from a 20-carbon polyunsaturated fatty acid, i.e. arachidonic acid. The rate-limiting enzymes for PG production are cyclooxygenase-1 and -2; however, which PG species such as PGE<sub>2</sub>, PGI<sub>2</sub>, or PGD<sub>2</sub> is produced by a given cell type is determined by specific synthases. PGD<sub>2</sub> is currently emerging as a key mediator capable of profoundly modulating eosinophil function, acting through two G-protein coupled receptors, D-type prostanoid receptor (DP) and chemoattractant receptor homologue expressed on Th2 cells (CRTH2). While the role of DP receptors has remained controversial, CRTH2 is considered as a major eosinophil-activating receptor with respect to eosinophil release from bone marrow, chemotaxis, oxidative burst and recruitment to sites of allergic inflammation. In contrast, PGE<sub>2</sub> seems to counteract eosinophil activation. Eosinophils express all four subtypes of E-type prostanoid receptors (EP1 through EP4), but only EP2 and EP4 appear to be involved in eosinophil modulation. Ligands for PGE<sub>2</sub> and PGD<sub>2</sub> receptors hence constitute important novel therapeutic targets for diseases that implicate pathological roles of eosinophils.

## **PAUL BERTICS LECTURESHIP**

William W. Busse

University of Wisconsin

Paul J. Bertics was the Robert Turell Professor of Biomolecular Chemistry at the University of Wisconsin School of Medicine and Public Health in Madison, WI. Paul died unexpectedly at age 55 years on December 22, 2011. My lecture will be in his honor and center on his contributions to the role of eosinophils in allergic inflammation. Paul Bertics contributed significantly to an understanding and appreciation that eosinophils are a heterogeneous population of cells, and, in particular, the biomolecular distinctions of cell populations in circulation and the airway, as well as their pattern of signal transduction. Using bronchoscopy and segmental antigen provocation with antigen, Paul Bertics characterized distinctions between the response of peripheral blood and airway eosinophils in activation, including IL-5, and their pattern of intracellular signaling and function. His work demonstrated that the recruitment of eosinophils to the airway from circulation was associated with a priming of this cell, and this change in phenotype was reflected in changes in expression of key intracellular pathways STAT3, STAT5a and STAT 5b as well as inflammatory function. Although these changes in activation may be related to alteration in IL-5R expression, his findings also suggested that the regulation of eosinophilic inflammatory processes are distinct when the cell is in circulation, where they are normally quiescent, versus the airway, where they are primed for activation and capable of generating an inflammatory response. Paul Bertics' work and collaborative research has added greatly to the appreciation and understanding of eosinophil biology in allergic inflammation in asthma and will be the focus of this presentation.

**PATHOGENESIS OF EOSINOPHILIC ESOPHAGITIS**

Marc E. Rothenberg

Director, Division of Allergy and Immunology, Director, Cincinnati Center for Eosinophilic Disorders, Cincinnati Children's Hospital Medical Center, Cincinnati OH 45229

Eosinophilic Esophagitis (EoE) is an emerging chronic gastrointestinal disorder characterized by marked esophageal eosinophilia that is etiologically linked to immune hypersensitivity to dietary antigens and persists from childhood into adulthood. EoE is growing in prevalence with rates as high as 1/1000 children, and is now the leading cause for proton pump inhibitor (PPI)-resistant dysphagia and food impaction in adults. The high response rate to food elimination diets, especially amino acid-based elemental diets, and the frequent recurrence of disease with food reintroduction imply that EoE is mediated by immune sensitization to foods. Indeed, we have shown that experimental EoE in mice can be induced by exposure to dietary, inhaled allergens or IL-13 via a variety of entry points including the skin, respiratory and gastrointestinal tracts. Consistent with an allergic etiology rather than an acid-induced esophagitis, swallowed glucocorticoids have been shown to be effective for the treatment of EoE and elicit local changes in gene expression in the esophagus, but there are substantial rates of glucocorticoid resistance and/or relapse while on therapy, necessitating a better understanding of drug resistance, relapse, and chronicity.

The molecular pathogenesis, genetics, immunological basis, and mechanism-based therapeutic intervention are presented.

Because the diseased tissue is readily obtained for scientific investigations, the study of EoE provides a unique opportunity to examine the etiology of eosinophilic diseases; as such, lessons learned will be applied to the field.



**EOSINOPHILS AND THE GUT MICROBIOME**

Sophie A. Fillon<sup>1</sup>, Joanne C. Masterson<sup>1</sup>, Ha Na Choe<sup>1</sup>, Caleb J. Kelly<sup>1</sup>, Shauna Schroeder<sup>1</sup>, J. Kirk Harris<sup>2</sup>, Brandie D. Wagner<sup>3</sup>, Rui Fang<sup>3</sup>, Mark Stevens<sup>2</sup>, Lindsay Hosford<sup>1</sup>, Rachel Harris<sup>1</sup>, Wendy Moore<sup>1</sup>, Alyson Yeckes<sup>1</sup>, Katie Amsden<sup>4</sup>, Amir F. Kagalwalla<sup>4</sup>, Charles E. Robertson<sup>5</sup>, Norman R. Pace<sup>5</sup>, James J. Lee<sup>6</sup>, Steven J. Ackerman<sup>7</sup> and Glenn T. Furuta<sup>1</sup>

Gastrointestinal Eosinophilic Diseases Program, Section of Pediatric Gastroenterology, Hepatology and Nutrition and Mucosal Inflammation Program, University of Colorado School of Medicine, Aurora, CO; Department of Pediatrics, National Jewish Health, Denver, CO Digestive Health Institute, Children's Hospital Colorado, Aurora CO<sup>1</sup>; Division of Pulmonary Medicine, Children's Hospital Colorado, University of Colorado, School of Medicine, Aurora, CO<sup>2</sup>; Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado, Aurora, CO<sup>3</sup>; Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, IL<sup>4</sup>; Molecular Cellular and Developmental Biology, University of Colorado Boulder, CO<sup>5</sup>; Mayo Clinic Scottsdale, S.C. Johnson Medical Research Center, Scottsdale, Arizona<sup>6</sup>; Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL<sup>7</sup>

**Background:** Reduction in bacterial diversity and dysbiosis of the gut microbiome contributes to gastrointestinal (GI) disease pathogenesis, particularly Inflammatory Bowel Diseases (IBD). The two most common forms of esophagitis are related to acid injury, Gastroesophageal Reflux Disease (GERD) and allergy, Eosinophilic Esophagitis (EoE). The underlying etiology of these GI diseases is not certain, but an emerging body of evidence implicates microbial triggering underlying GI inflammation. Eosinophil presence is increased in IBD and EoE tissues and eosinophils generate different molecules with anti-microbial properties. We hypothesize that eosinophils modulate the gastrointestinal microbiota. The aim of this study was to determine the eosinophils impact on the composition of the intestinal microbiota.

**Methods:** Two strains of eosinophil deficient mice (PHIL and  $\Delta$ dbl-GATA) and their corresponding wild type background (C57BL/6 and BALB/c), were studied. Mice were housed under specific pathogen free conditions. Between 8-12 weeks of age, C57BL/6 (n=12) and PHIL (n=9) female and BALB/c (n=13) and  $\Delta$ dbl-GATA (n=13) female mice stool pellets were collected for DNA isolation (Qiagen). For the human studies, nasal swab, oral and esophageal string test samples and mucosal biopsies were collected from EoE subjects with symptoms of esophageal dysfunction, esophageal eosinophilia (>15 eosinophils per high power field), EoE subjects who had undergone at least 8 weeks of treatment (topical steroids or dietary elimination) (n=37), GERD subjects with symptoms of vomiting or heartburn that had responded to proton pump inhibition and/or had an abnormal pH impedance monitor of the distal esophagus (n=8), and normal controls (n=25). Composition of the microbiota in the mouse or human samples was determined by metagenomic analysis of PCR amplified bacterial 16S rDNA using 454 pyrosequencing, and phylogenetic analysis.

**Results:** Pyrosequencing data revealed that bacterial genera *Shigella* and *Enterobacter* were uniquely present in the stool from PHIL mice but not in C57BL/6, and that *Alistipes*, *Odoribacter* and *Limibacter* were uniquely present in the stool from  $\Delta$ dbl-GATA mice but not in BALB/c. The microbiota on esophageal biopsies and strings from healthy controls and subjects with esophagitis included similar overall panels of bacterial genera. A different microbiota was observed in the nose and the oral cavity as compared to the esophagus. Subjects with EoE had a significant increase in *Haemophilus*, and subjects with GERD had a decrease in *Streptococcus* in comparison to normal esophageal microbiota.

**Conclusions:** Eosinophils participate in the modulation of the gastrointestinal microbiota, and the absence of eosinophils increases the susceptibility of mice to colonization by opportunistic pathogens based on the presence of *Shigella* and *Enterobacter* in the PHIL mice and *Alistipes*, *Limibacter* and *Odoribacter* in the  $\Delta$ dbl-GATA mice. Esophageal inflammation in humans is associated with alteration of the esophageal microbiome with an increase of esophageal *Haemophilus* in EoE and a decrease of *Streptococcus* in GERD.

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**STEROIDS VERSUS ALLERGEN AVOIDANCE IN THE MANAGEMENT OF EOSINOPHILIC ESOPHAGITIS (EOE)**

Alex Straumann

Swiss EoE Clinic and EoE Research Network, Olten, Switzerland

Eosinophilic esophagitis (EoE) is a chronic, Th2-type inflammatory disease of the esophagus.

Soon after the first recognition of EoE it was speculated that the eosinophilic inflammation of the esophageal epithelium could be the result of an allergic response to ingested food. As a consequence, several attempts were undertaken to protect the esophageal surface from potential allergens: The first dietary approach consisted of a completely protein-free nutrition by means of an amino-acid formula (elemental diet). Elemental diet was indeed highly efficient in children bringing more than 70% of patients with active EoE within 6 weeks in remission. A second approach used an individual-targeted elimination diet, based on allergy testing. This, individual-adjusted diet showed some efficacy in children, but had no convincing effects in adults. Lastly, an empiric approach was developed avoiding the six most critical food categories without respecting the allergic state of an individual. This so called 6-food elimination diet (6-FED) based on the avoidance of milk, wheat, eggs, nuts, soya and sea-food, was effective in children and in adults and brought up to 70% of patients having active EoE in remission. Unfortunately, after cessation of any type of dietary restriction all patients relapsed rapidly. Neither elemental diet, individual-targeted elimination diet nor empiric 6-food elimination diet were able to cure EoE. Further drawbacks of these allergen-avoidance strategies are that 1.) All these diets have a strong negative impact on the quality of life of the patients; and 2.) All open-label trials evaluating the efficacy of diets were performed by highly motivated research teams.

EoE has several similarities with allergic asthma. In parallel with the evaluation of allergen avoidance strategies, several drugs with established efficacy in asthma were evaluated in EoE. Corticosteroids (CS) – systemically (SCS) and topically administered (TCS) – had shown convincing efficacy in several randomized, placebo-controlled clinical trials. SCS and TCS were similar effective bringing approximately 70% of adult and pediatric patients with active EoE successful in remission. As could be expected, SCS had much more side effects than TCS. Unfortunately, after cessation of the medication all patients relapsed rapidly. Neither treatment with SCS nor with TCS was able to cure EoE.

So far, no controlled prospective trial has been performed comparing the dietary allergen-avoidance strategy with the CS-treatment. The decision whether a dietary or a medical approach is in a given situation preferable depends therefore currently on the patient's desire and on the local habits.

**STATE OF THE ART: EOSINOPHILS AND HOST DEFENSE**

Kimberly D. Dyer and Helene F. Rosenberg

Inflammation Immunobiology Section, Laboratory of Allergic Diseases, NIAID, NIH, Bethesda, MD, U.S.A.

Traditionally, eosinophils have been associated with host defense against helminth infections. However, it is becoming clear that eosinophils play a more substantial and diverse role in host defense than was previously appreciated. Eosinophils have been implicated in host defense against numerous pathogens other than helminths, including bacteria, fungi, and viruses.

The original perception that eosinophils promote host defense against helminths arose from co-localization of eosinophils and parasites in tissue specimens. Animal studies have generated mixed and inconsistent support for this hypothesis<sup>1</sup>. Likewise, a recent analysis of ECP coding sequence polymorphisms in human populations suggested that the role of eosinophils may relate to the contribution to liver damage rather than to a direct role in host defense against the *Schistosoma mansoni* pathogen<sup>2</sup>. Interestingly, recent findings suggest that, in specific situations, eosinophils may serve to support parasite survival. Eosinophils were found to be essential for the development of nurse cells, a specialized myocyte that develops in response to infection with *Trichinella spiralis* that is crucial for support of larval growth and development<sup>3</sup>.

Although the eosinophil cationic granules proteins major basic protein (MBP) and ECP were previously characterized as bactericidal *in vitro*, the role of eosinophils in anti-bacterial host defense *in vivo*, specifically, promoting bacterial clearance and survival in mouse models of gram-negative sepsis, has only recently been elucidated<sup>4,5</sup>. MBP, ECP and mitochondrial DNA were described as components of eosinophil-derived extracellular traps (ETs), antibacterial structures released in “catapult” fashion from cytokine-primed eosinophils in response gram-negative lipopolysaccharide, similar to those described from neutrophils<sup>4</sup>.

Fungal extracts stimulate degranulation of human eosinophils<sup>6</sup> via interactions with protease activated receptor (PAR)-2. MBP and EDN are released into the medium and adhere to the fungal surface, interactions that result in contact-dependent cytotoxicity<sup>7</sup>. Eosinophils themselves can adhere to fungal beta-glucan via cell-surface CD11b<sup>8</sup>. It is not clear what role the fungal-dependent eosinophil activation, degranulation and adherence may play *in vivo*, and whether these findings promote host defense or allergic phenomena.

Several studies have documented the interactions between eosinophils and respiratory viruses, and have suggested that eosinophils may limit virus infectivity and promote virion clearance<sup>9-11</sup>. Recently, Percopo et al.<sup>12</sup> evaluated antiviral responses to pneumonia virus of mice (PVM) in two mouse models of Th2-driven eosinophilic airway inflammation, and found that degranulating eosinophils not only promote a substantial reduction in virus titer, but that they also protect mice from the otherwise lethal sequelae of PVM infection.

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**EOSINOPHILS ARE PIVOTAL MEDIATORS OF DISTINCT IMMUNE MECHANISMS IN PRIMARY VERSUS SECONDARY NEMATODE INFECTION**

Lu Huang<sup>1</sup>, Nebiat G. Gebreselassie<sup>1</sup>, Lucille F. Gagliardo<sup>1</sup>, Nancy A. Lee<sup>2</sup>, James J. Lee<sup>3</sup>, and Judith A. Appleton<sup>1</sup>

<sup>1</sup>Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 USA. <sup>2</sup>Department of Hematology and Oncology and <sup>3</sup>Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259 USA.

The eosinophil is a defining cellular feature of the immune response to parasitic worm infection. Research into the role of eosinophils in host defense has produced findings that appear contradictory. Eosinophil-mediated toxicity for nematode larvae was documented many years ago, while recent work has revealed that eosinophils promote survival and/or growth of larvae and worms. We have found two distinct and opposite influences of eosinophils in primary versus secondary infection of mice with the nematode, *Trichinella spiralis*. This study employed targeted gene knock-out and transgenic mice, together with adoptive transfer protocols, to assess eosinophil-mediated effects on immune function, parasite burden, host vitality, and larval growth. The worm completes its life cycle in a single host, first colonizing the intestine and then releasing larvae that infect skeletal muscle. In neither primary nor secondary infection did eosinophils influence the survival or reproduction of intestinal worms. In contrast, during primary infection, eosinophils preserved muscle stage larvae by promoting immunity mediated by myeloid dendritic cells, IL-4/IL-10 secreting Th2 cells, and M2 macrophages. During secondary infection, eosinophils contributed to clearance of larvae in an antibody dependent manner, most likely affecting larvae as they migrated, prior to colonization of muscle. Thus, the eosinophil is a pivotal mediator of distinct immune mechanisms that protect larvae during primary infection and clear them during secondary infection. Supported by DHHS grants AI081043 and AI097555.

**STRUCTURAL ELEMENTS OF THE EOSINOPHIL CATIONIC PROTEIN IMPORTANT IN HOST DEFENSE**

Ester Boix<sup>1</sup>, Marc Torrent<sup>1</sup>, David Pulido<sup>1</sup>, Vivian Salazar<sup>1</sup>, Jose A. Blanco<sup>1</sup>, M. Moussaoui<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Biosciences Faculty, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain

**Background:** The human eosinophil cationic protein (ECP) is stored in the eosinophil secondary granule matrix and is released upon the eosinophil activation during infection and inflammatory processes. ECP toxicity has been reported against bacteria, viruses, protozoa and helminths. Although the protein, also named RNase 3, belongs to the vertebrate RNase superfamily, most of its described antipathogen properties were found unrelated to its catalytic activity. So far, despite all the applied efforts to tackle the protein behavior, little can still be ascertained upon its real physiological role during innate immunity. In this work we have committed ourselves to unravel the structural determinants for the protein antimicrobial activity.

**Methods:** Structural determinants for ECP antimicrobial activities were screened by prediction tools, proteolysis, peptide synthesis and site –directed mutagenesis. The protein toxicity was tested against a variety of Gram negative and Gram positive bacteria species. ECP mechanism of action was explored using bacteria cultures and synthetic membrane models.

**Results:** Experimental data combined with in silico predictions were applied to define the main protein regions involved in the antimicrobial activities. Key motives were located for the protein self aggregation, cell agglutination, cell surface binding and membrane interaction. ECP structural –functional relationship analysis is addressed as a reference study model to define the requirements that may confer protein sequences to acquire a host defense function. The particular ECP properties are also discussed within the context of the vertebrate RNase superfamily lineage.

**Conclusions:** Structural analysis of ECP antimicrobial activity suggests that this eosinophil secretion protein performs a host defense role against potential human pathogens. The small cationic protein could work as a multitask weapon targeting simultaneously the invading microorganisms at distinct cellular levels. The identified antimicrobial motives can serve as lead templates for the engineering of novel antibiotics.

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## ANTI-IL-5 MONOCLONAL ANTIBODIES AS A NEW CLASS OF DRUGS FOR THE TREATMENT OF ASTHMA

Andrew Wardlaw

Institute for Lung Health, Department of Infection Immunity and Inflammation, University of Leicester UK

IL-5 is a selective growth factor that is essential for expansion of the eosinophil lineage. In humans the IL-5 receptor is only expressed on eosinophils, basophils and a small population of bone marrow progenitors. The IL-5 pathway is therefore an ideal therapeutic target for strategies aimed at reducing eosinophilic inflammation. Two drugs, both humanised monoclonal antibodies which bind to IL-5 and prevent it binding to its receptor, have reached clinical trials; Mepolizumab and Reslizumab (previously SCH55700). Initial studies with these drugs in asthma, where most of the drug development has been targeted, were disappointing in that despite a marked reduction in blood, sputum and BAL eosinophils there was no effect on FEV1 (either after allergen challenge or in clinical asthma), airway hyperresponsiveness (AHR) or symptoms (1-3). Moreover the drug was only partially effective in reducing the number of eosinophils in the bronchial submucosa (4). However there was no selection for eosinophilic patients in these studies so any effect may have been diluted by including people who did not have a significant airway eosinophilia. In addition the evidence from a large number of studies suggests that eosinophils play a limited role in the abnormal airway smooth muscle (ASM) function which defines asthma and is responsible for the classical day-to-day symptoms. Eosinophils are much more closely associated with severe exacerbations. When Mepolizumab was investigated in eosinophilic patients with a propensity to severe exacerbations it was effective both in reducing the number of severe exacerbations in patients on fixed treatment and in preventing their occurrence during reduction of oral steroid treatment (5, 6). The findings of the study by Haldar et al have recently been confirmed in a multi-centre study undertaken by GSK. (7). Interestingly in this study the lowest dose of Mepolizumab was effective in reducing exacerbations despite a limited effect on the sputum eosinophilia although the peripheral blood eosinophilia remained suppressed. Thus the benefit of Mepolizumab appears to persist irrespective of a marked effect on the number of lung eosinophils so the exact mechanism by which it is working is not certain not least because it only has a limited effect on markers of eosinophil activation. Reslizumab is currently being investigated in an ambitious series of large multi-centre clinical trials being undertaken by TEVA. They had previously investigated Reslizumab in a phase 2 study. They recruited 88 moderate eosinophilic asthmatics to a four-month double blind placebo controlled study with improvement in asthma control score (ACQ) as the primary outcome. They confirmed the expected reduction in blood and sputum eosinophils. Improvements in ACQ just failed to reach significance, but there was a significant improvement in FEV1 in the active group (8). There were insufficient exacerbations to make any comment on this outcome although a 50% reduction was seen in the active group (9). In summary anti-IL-5 monoclonal antibodies show great promise as new types of biological therapies for the effective prevention of exacerbations in severe eosinophilic asthma

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**RESULTS FROM TWO CLINICAL STUDIES OF BENRALIZUMAB, AN ANTI-INTERLEUKIN-5 RECEPTOR ALPHA MONOCLONAL ANTIBODY: EFFECTS ON AIRWAY EOSINOPHILS AND ACUTE ASTHMA EXACERBATIONS**

Donald G. Raible

MedImmune LLC, Gaithersburg, MD 20878, USA

**Background:** Benralizumab (MEDI-563) targets the interleukin-5 receptor  $\alpha$ , depleting eosinophils through enhanced antibody-dependent cell-mediated cytotoxicity. Two clinical studies were conducted: study 1 to better understand the effects of benralizumab on airway eosinophils; and study 2 to determine if benralizumab can decrease asthma exacerbations.

**Methods:** In study 1, 27 subjects with eosinophilic asthma (sputum eosinophils  $>2.5\%$ ) were randomized to either 1 IV dose of placebo or benralizumab (1.0 mg/kg), or 3 monthly SC injections of placebo or benralizumab (100 or 200 mg). The IV and SC cohorts were consecutive. Airway biopsies were obtained prior to day 0 and 28 days after the last dose. Tissue eosinophils/mm<sup>2</sup> were counted from at least 4 airway biopsies per subject (range 4-6) and the results averaged.

In study 2, subjects with  $\geq 1$  previous asthma exacerbation who presented to the emergency department with an asthma exacerbation and had partial response to standard therapy received either 1 IV infusion of placebo (n=38) or benralizumab (0.3 mg/kg, n=36; 1.0 mg/kg, n=36) added to outpatient care. The primary outcome was the proportion of subjects with  $\geq 1$  exacerbation at 12 weeks in placebo vs. the combined benralizumab groups. Other outcomes included the time-weighted rate of exacerbations at week 12, adverse events, and blood eosinophil counts.

**Results:** In study 1, 27 subjects provided airway biopsies. Airway mucosal/submucosal eosinophil counts decreased from screening to endpoint for most subjects who received benralizumab (IV cohort: 6/8, 75.0%; SC cohort: 8/9, 88.9%). For the combined placebo cohorts (n=10), there was a 4.7% increase in airway mucosal/submucosal eosinophils 28 days after the last dose (interquartile range [Q1-Q3], -64.1% to +84.3%) compared with -83.1% (Q1-Q3: -95.8% to -57.6%) for the combined benralizumab cohorts (n=17). The difference between placebo vs. benralizumab was significant ( $P=.02$ ).

In study 2, 108 of the 110 subjects were evaluable. Over 12 weeks, the proportion of subjects experiencing  $\geq 1$  exacerbation was not different between placebo and the combined benralizumab groups (38.9% vs. 33.3%,  $P=.67$ ). However, the weighted rate of exacerbations was reduced by 49% (3.59 vs. 1.82;  $P=.01$ ) and rates of exacerbations resulting in hospitalization by 60% (1.62 vs. 0.65;  $P=.02$ ). Benralizumab reduced peripheral blood eosinophil counts but did not significantly affect other outcomes.

Adverse events (AE) were similar between placebo and benralizumab in both studies. One non-drug-related serious AE occurred in study 1; and in study 2, serious AEs considered related to benralizumab were pyrexia (n=2), tachycardia (n=1), and anxiety (n=1). In study 1, the percent of subjects with drug-related AEs was: 0% placebo vs. 37.5% benralizumab in the IV cohort, and 20% placebo vs. 11.1% benralizumab in the SC cohort. In study 2, AEs in  $>5\%$  of subjects who received benralizumab were asthma, headache, dizziness, cough, and fever.

**Conclusions:** These data suggest that the anti-interleukin-5Ra antibody, benralizumab, depletes airway tissue eosinophils. In addition, the asthma exacerbation rate in subjects presenting to the emergency department was decreased. Longer studies in a broader asthma population are needed to understand the potential for benralizumab in inadequately controlled asthma.

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**TREATMENT OF ADULT ASTHMATICS WITH THE ANTI-IL13 LEBRIKIZUMAB**

John G. Matthews,<sup>1</sup> on behalf of the MILLY study group

<sup>1</sup>Genentech Inc., South San Francisco, CA, USA

**Background:** Asthma is a chronic respiratory disorder with considerable heterogeneity in its clinical course and response to treatment. There is compelling evidence that interleukin 13 (IL13) plays a pivotal role in the pathogenesis of type 2 helper T-cell (Th2)-driven asthma and that IL13 blockade represents a potential therapy option for severe asthma.

**Methods:** A randomized, double-blind, placebo-controlled study evaluated lebrikizumab, a humanized anti-IL13 monoclonal antibody, in 219 adults receiving inhaled corticosteroids with uncontrolled asthma has been published. Patient subgroups were prespecified according to baseline Th2 status and serum periostin levels. Primary efficacy outcome was relative change in forced expiratory volume in 1 sec (FEV1) from baseline to Week 12. Secondary and exploratory outcomes included severe exacerbation rates at Week 24 and Week 32, respectively.

**Results:** At Week 12, the mean increase in FEV1 was higher in the lebrikizumab group vs placebo (9.8% vs 4.3%;  $P=0.02$ ). These improvements were highest in periostin-high patients (lebrikizumab 14.0%, placebo 5.8%;  $P=0.03$ ). Among periostin-low patients, the increase from baseline FEV1 was not statistically different between groups (lebrikizumab 5.1%, placebo 3.5%;  $P=0.61$ ). Severe exacerbation rates at Week 24 were reduced by 43% in the lebrikizumab group vs placebo ( $P=0.10$ ). In periostin-high patients, lebrikizumab reduced severe exacerbation rates by 67% vs placebo ( $P=0.08$ ), whereas in periostin-low patients, lebrikizumab reduced severe exacerbation rates by 29% ( $P=0.44$ ). At Week 32, severe exacerbation rates were reduced by 50% in the lebrikizumab group vs placebo ( $P=0.03$ ), with larger reductions in periostin-high patients (61%;  $P=0.06$ ) vs periostin-low patients (43%;  $P=0.18$ ). Adverse event profiles were similar in both groups (lebrikizumab 74.5%, placebo 78.6%).

**Conclusions:** Lebrikizumab produced significant improvements in mean FEV1 and had a trend for a reduced severe exacerbation rate vs placebo. Periostin-high patients showed numerically greater improvements in FEV1 and reduction in severe exacerbation rates following lebrikizumab than periostin-low patients. These data support that periostin may be a useful biomarker identifying patients more likely respond to lebrikizumab.

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**HUMAN AND MOUSE EOSINOPHILS: ISOLATION, GENERATION AND RELATED APPLICATIONS**

Helene F. Rosenberg<sup>1</sup>, Caroline M. Percopo<sup>1</sup>, Kimberly D. Dyer<sup>1</sup>, Eva M. Sturm<sup>1,2</sup>

<sup>1</sup>Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA and <sup>2</sup>Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

**Background:** Eosinophils represent only a fraction of the ongoing hematopoietic activity in bone marrow at any given time, even under moderate Th2 stimulation. Prior to the introduction of antibody-based selection methods, eosinophils isolation was based primarily on discontinuous Percoll gradients, which were difficult, time-consuming and yielded erratic results. In addition to direct isolation, mouse and human eosinophils can be generated *ex vivo* in cytokine-supported progenitor cultures, although these methods can result in varying degrees of phenotypic and functional maturity and purity.

**Results/Conclusions:** We will present current methods used to isolate human and mouse eosinophils [1, 2], and we will compare and contrast these methods and their outcomes to those used to generate both human and mouse eosinophils from selected (CD34+) and unselected bone marrow progenitors in tissue culture [3 - 5]. We will provide some insight into recent applications of these latter methods, including reconstitution in response to allergen challenge [6] and *in vivo* chemotaxis assays [7]. Overall, our intent is to discuss the appropriate use of these methods, to provide information on suitable interpretations and to share troubleshooting strategies.

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**EOSINOPHILS ARE ESSENTIAL FOR THE LONG-TERM SURVIVAL OF PLASMA CELLS**

Chu Van Trung and Claudia Berek

German Rheumatism Research Center, Institut der Leibniz Gemeinschaft, Berlin

Max Delbrück Center for Molecular Medicine, Berlin Long-lived plasma cells and their antibodies are an essential part of immune protection. In some situations, such as in autoimmune diseases, however, antibodies may be detrimental to the organism. Long-lived plasma cells survived in specialized niches in the bone marrow where long-term maintenance of these cells is ensured by survival factors. However, little was known about the cellular sources of cytokines required for the survival of plasma cells. In aim of the study, we demonstrated that bone marrow eosinophils are the key providers of plasma cell survival factors such as APRIL and IL-6. In eosinophil-deficient mice, the accumulation of plasma cells in the bone marrow is impaired. Only after reconstitution with eosinophils, significant numbers of plasma cells are found in the bone marrow of these mice. Moreover, depletion of eosinophils induced apoptosis in long-lived plasma cell compartment. These findings demonstrate that eosinophils are required for the retention and long-term maintenance of plasma cells in the bone marrow. Thus, it becomes possible to directly target the long-lived plasma cells in the bone marrow by destroying the components of plasma cell niches. These finding opens new ways to treat antibody-mediated autoimmune diseases which B cells are depleted. However, a specific depletion of mature plasma cells so far is not possible. Using eosinophil specific antibodies for the first time it is possible to directly attack the long-lived plasma cells in the bone marrow.

**EHRlich AWARD LECTURESHIP: EOSINOPHIL LIPID BODIES FROM ENIGMAS TO EICOSASOMES AND MORE**

Peter F. Weller<sup>1</sup>

<sup>1</sup>Divisions of Infectious Diseases and Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.

**Background:** Ehrlich's cardinal applications of novel staining techniques with alcohol-based aniline dyes, including eosin, enabled him to identify eosinophils as a distinct leukocytic granulocyte. Subsequently over more than 100 years, the use of alcohol-based hematologic stains led progressively to the recognitions of the associations of eosin-granule staining eosinophilic granulocytes within the blood and tissues with varied host responses and diseases. While conventional alcohol-based hematologic stains could differentiate and identify eosin-staining granules within eosinophils, an untoward consequence of the use of hematologic staining protocols was that lipid-rich organelles within eosinophils (and other leukocytes and cells) were dissolved with alcohol-based staining. With alternative methods of cell preparation and staining, including the use of osmium that fixed and preserved lipids as employed for electron microscopy, distinct osmiophilic intracellular structures, termed lipid bodies, were revealed and recognized to be increased in eosinophils (and other leukocytes and cells) associated with varied forms of inflammation and diseases. Were eosinophil lipid bodies "granules," how do lipid bodies form, what functions and roles do lipid bodies play in eosinophils, and do studies of eosinophil lipid bodies provide insights into the functions of these organelles in other leukocytes and cells?

**Methods:** Investigations into the structure and functions of eosinophil lipid bodies have been advanced progressively over many years by the critical collaborative contributions of many junior and senior co-investigators. Multiple experimental approaches have been applied including immunofluorescence and cytochemical microscopy as well as immunogold electron microscopy to localize key eicosanoid synthesizing enzymes at eosinophil lipid bodies. Novel techniques were developed to fix newly synthesized eicosanoids so that these eicosanoids could be immunolocalized at their intracellular sites of synthesis. Additional electron microscopy studies were applied to probe the ultrastructure of eosinophil lipid bodies.

**Results:** We documented that eosinophil lipid bodies were formed by distinct cell signaling responses, that eosinophil lipid body formation correlated with increased eosinophil eicosanoid formation, that eosinophil lipid bodies were sites of localization of key eicosanoid-synthesizing enzymes and were specific sites of eicosanoid synthesis, that lipid body-derived eicosanoids exerted intracrine signaling activities and that eosinophil lipid bodies were ultrastructurally distinct from adipocyte lipid droplets.

**Conclusion:** Investigations past and ongoing have taken lipid bodies within eosinophils from intracellular structures of uncertain formation and function to their recognition as sites of eicosanoid formation ("eicosomes"). Notably, these investigations have been based on critical experimental contributions of numerous junior and senior colleagues. I fully recognize each of my colleagues as co-investigators without whose prior and ongoing research studies we would not have learned as much as we know about lipid bodies as organelles. It is with much pride and enormous personal satisfaction that I can acknowledge my collegial co-investigators. Our collective focus on defining the structure and functions of lipid bodies within eosinophils has enabled a broader recognition of the roles of lipid bodies in other leukocytes and cells as "eicosomes."

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## **HYPEREOSINOPHILIC DISEASE: END ORGAN MANIFESTATIONS: ARE THEY USEFUL FOR DIFFERENTIAL DIAGNOSIS?**

Gerald J. Gleich

The recent classification\* of hypereosinophilic disease (also referred to as the hypereosinophilic syndrome) (HES) defines HES as: 1. Eosinophilia with  $>1.5$  eosinophils  $\times 10^9/L$  blood on 2 determinations (interval  $>1$  month). 2. Related organ damage, and 3. Absence of an alternative explanation for the observed organ damage. Eosinophil-related organ damage consists of eosinophil infiltration and/or marked deposition of eosinophil granule proteins associated with organ dysfunction and with various clinical presentations, including: (1) fibrosis (e.g., lung, heart, digestive tract, skin, and others); (2) thrombosis with or without thromboembolism; (3) cutaneous and/or mucosal erythema, edema/angioedema, ulceration, or eczema; (4) peripheral or central neuropathy with chronic or recurrent neurological deficit; and (5) less common organ manifestations of HES involving liver, pancreas, kidney, and others. Hypereosinophilia (HE) ( $>1.5$  eosinophils  $\times 10^9/L$  blood, as defined above) can also accompany single-organ diseases, such as asthma or gastroenteritis. Thus, patients with HE can present with protean manifestations and involvement of virtually any organ. Yet the clinical presentation often gives important clues about the diagnosis and pathophysiology. In this presentation, I summarize rough guidelines for associating clinical presentations and disease classifications based on organ involvement.

Clinical presentations include: 1. Thromboembolism with infarction of limbs and, more commonly, the central nervous system (usually a hallmark of myeloproliferative HES with cardiac involvement). 2. Dyspnea with wheezing (eosinophilic asthma is remarkably common and under-appreciated by physicians; consider chest x-ray/CT to exclude pulmonary infiltration). 3. Anemia (often indicative of myeloproliferative neoplasm/myelodysplastic syndrome but can be associated with iron deficiency and protein-losing enteropathy). 4. Gastroenteric symptoms, such as emesis, diarrhea, abdominal pain or dysphagia (most commonly associated with eosinophilic gastroenteritis. Also test for food allergy and determine if fecal  $\alpha 1$ -anti-trypsin is increased. Consider eosinophilic granulomatosis with polyangiitis/Churg-Strauss syndrome that can present with abdominal pain that may be a warning of impending intestinal infarction. Presently, eosinophilic esophagitis is a common cause of dysphagia but is not typically accompanied by HE). 5. Skin manifestations including eczematous and edematous lesions (broad differential diagnosis that includes atopic dermatitis most commonly and, less commonly, eosinophilic cellulitis/Wells' syndrome, eosinophilic fasciitis/Shulman's syndrome, bullous pemphigoid, drug reaction with eosinophilia and systemic symptoms/DRESS, episodic angioedema with HE and others). 6. HE on a routine blood count (this may be benign or a harbinger of HES and both warrant careful following with assessments for evolving organ involvement). 7. Peripheral neuropathy (most commonly eosinophilic granulomatosis with polyangiitis/Churg-Strauss syndrome or idiopathic HES).

\* Valent P et al. *J Allergy Clin Immunol* 2012;130:607-12

## **HYPEREOSINOPHILIC SYNDROME AS A MULTIDISCIPLINARY DISORDER**

Grzegorz Helbig

Department of Hematology and Bone Marrow Transplantation, Silesian Medical University

Eosinophils play an important role in the pathogenesis of various disorders. The detrimental effect of hypereosinophilia (HE) is associated with inflammation, tissue fibrosis and tendency to thrombosis and these processes may impair the function of critical organs e.g. heart, lungs or central nervous system. Typically eosinophils in peripheral blood represent less than 5% of leukocytes and therefore eosinophilia is defined as absolute blood eosinophil count (AEC) exceeding  $0.5 \times 10^9/L$  whereas HE refers to AEC greater than  $1.5 \times 10^9/L$ . Tissue eosinophilia may be accompanied or not by blood eosinophilia and vice versa. The presence of a modest number of eosinophils is found in normal tissue e.g. gastrointestinal tract, their excessive infiltration may result in organ damage and lead to local or systemic disease. In the light of our current knowledge the already existing criteria of HE-related disorders possess certain limitations and require the re-evaluation. A panel of multidisciplinary experts agreed on unifying terminology, definition and criteria of HE-related conditions. A new classification distinguishes several variants and takes into account hematologic and laboratory findings as well as the underlying cause of HE. The expert panel proposed the term HE of undetermined significance for patients with benign HE, however its clinical implication remains unclear. This lecture will summarize the contemporary consensus on classification of HE. It discusses the most common clinical manifestations of HE as well as presents the therapeutic options for different HE variants. Finally, I will share my experience in the field of HE.

**ESTABLISHMENT OF AN INTERNATIONAL NETWORK OF INVESTIGATORS OF EOSINOPHIL ASSOCIATED MYELOPROLIFERATIVE DISORDERS**

Peter Valent

Eosinophil disorders and related syndromes are a heterogeneous group of conditions characterized by marked persistent blood eosinophilia and involvement of one or more organ systems. The hypereosinophilic (HE) state is defined by a persistent eosinophil count exceeding  $1.5 \times 10^9$ /liter blood. Several different neoplastic, paraneoplastic, infectious, and allergic disorders may underlie HE. Eosinophil-induced organ damage with more or less typical symptoms may develop in these patients. The final diagnosis is based on clinical, molecular and histopathological criteria, and the presence of signs and symptoms indicative of HE-induced organ damage, the latter often manifesting as hypereosinophilic syndrome (HES). The clinical course, prognosis, and response to certain drugs vary greatly among patients and among disease-variants. During the past few years, several new markers and targets have been identified, improving diagnosis, prognostication and therapy for patients with HE-related disorders. Moreover, several attempts have been made to establish robust disease-related criteria and a global classification for HE-related diseases. However, the pathogenesis and mechanisms of HE and of HE-induced organ damage are complex, and expert opinions remain divided. The ICOG-EO group has recently proposed an updated consensus classification on eosinophil disorders with robust disease-related criteria. In this proposal, underlying diseases and neoplasms are delineated from hypereosinophilic syndromes, characterized by a unique and clinically relevant organ damage.

Eosinophil disorders and related syndromes are a heterogeneous group of conditions characterized by marked persistent blood eosinophilia and involvement of one or more organ systems. The hypereosinophilic (HE) state is defined by a persistent eosinophil count exceeding  $1.5 \times 10^9$ /liter blood. Several different neoplastic, paraneoplastic, infectious, and allergic disorders may underlie HE. Eosinophil-induced organ damage with more or less typical symptoms may develop in these patients. The final diagnosis is based on clinical, molecular and histopathological criteria, and the presence of signs and symptoms indicative of HE-induced organ damage, the latter often manifesting as hypereosinophilic syndrome (HES). The clinical course, prognosis, and response to certain drugs vary greatly among patients and among disease-variants. During the past few years, several new markers and targets have been identified, improving diagnosis, prognostication and therapy for patients with HE-related disorders. Moreover, several attempts have been made to establish robust disease-related criteria and a global classification for HE-related diseases. However, the pathogenesis and mechanisms of HE and of HE-induced organ damage are complex, and expert opinions remain divided. The ICOG-EO group has recently proposed an updated consensus classification on eosinophil disorders with robust disease-related criteria. In this proposal, underlying diseases and neoplasms are delineated from hypereosinophilic syndromes, characterized by a unique and clinically relevant organ damage.

**CLINICAL MANIFESTATIONS AND BIOMARKERS OF EOSINOPHIL ASSOCIATED MYELOPROLIFERATIVE DISEASE**Jean Emmanuel Kahn, MD-PhD

Department of Internal Medicine - Hôpital Foch - 40 rue Worth, 92151 Suresnes Cedex – France. Tel + 33 1 46 25 25 79, je.kahn@hopital-foch.org; French Eosinophil Network – Immunology Laboratory - EA2686 - CRHU de Lille - Pole Recherche - I Place de Verdun, 59045 Lille Cedex – France. Tel +33 3 20 62 68 61

A small proportion of chronic unexplained hypereosinophilia is related to a clonal expansion of eosinophil lineage. According to WHO classification of myeloproliferative neoplasms and to recent classification of eosinophilic disorders, they are now considered as chronic eosinophilic leukemia (CEL) or primary/neoplastic hypereosinophilia (HE<sub>N</sub>), in which *FIP1L1/PDGFR $\alpha$*  represents the most frequent clonal event identified.

Eosinophil infiltration of all tissue and organ is observed in HE<sub>N</sub> as in others variants of hypereosinophilic syndromes and cannot consequently be sufficient to characterize HE<sub>N</sub>. Nevertheless, some clinical or biological features are very suggestive of HES<sub>N</sub>. All series published worldwide confirmed the adult-male predominance in HES<sub>N</sub>, especially in *F/P+* CEL, with less than 10 *F/P+* female, and less than 5 children described to date.

Prevalence of cardiac involvement, particularly endomyocardial fibrosis, appears higher in HE<sub>N</sub> than in reactive HES. In a recent national cohort of 44 patients with *F/P+* CEL, 15 patients (34%) with a specific eosinophilic cardiac disease, including endomyocardial fibrosis (n=7) were identified. Interestingly, although only two patients need cardiac surgery, no cardiac-related death occurred in this cohort. Similarly to reactive HES (HES<sub>R</sub>) cutaneous manifestations are observed in two third of patients. While mouth ulcers seem very suggestive of HES<sub>N</sub>, angioedema, a common manifestation of lymphoid variant of HES, is unfrequent in these patients. The other most common features in *F/P+* CEL are splenomegaly (52%), pulmonary manifestations (46%), while gastro-intestinal or central nervous system involvements seem less frequent (less than 20%).

As seen in most myeloid neoplasms, anemia and thrombocytopenia are frequently observed in HES<sub>N</sub>, while uncommon in HES<sub>R</sub>. Elevated B12 and/or tryptase levels associated with normal levels of total IgE is the common biological picture of HES<sub>N</sub>, especially in *F/P+* CEL. In the absence of a clonal event identified, such biological profile may also help to identify patient who could respond to tyrosine kinase inhibitors. WT1, a surrogate marker of myeloid neoplasms, may also help to identify HES<sub>N</sub>, but conflicting results have been published. Beside *F/P* fusion gene, which represents probably at least 80% of HES<sub>N</sub>, other clonal events are represented by translocations involving *PDGFRB* (especially ETV6-PRGFRB) and *FGFR1* genes, *JAK2* and *PDGFR $\alpha$*  gain-of-function mutations.

Some common clinical and biological findings may thus help clinicians to identify HES<sub>N</sub>. An early diagnosis is necessary in this variant of HES, which harbor probably the worse prognosis in the absence of an appropriate treatment (especially imatinib in *F/P+* CEL).

**THE TRANSCRIPTION FACTOR XBP-1 IS A CRITICAL REGULATOR OF EOSINOPHIL DEVELOPMENT**

Sarah E. Bettigole<sup>1</sup>, Lisa A. Spencer<sup>2</sup>, Peter F. Weller<sup>2</sup>, and Laurie H. Glimcher<sup>1</sup>

<sup>1</sup> Department of Medicine, Weill Cornell Medical College, New York, NY 10065, USA

<sup>2</sup> Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

**Background:** Numerous studies have documented an important role for the endoplasmic reticulum stress response transcription factor XBP-1 in regulating the development of a variety of secretory and granule-containing cells. However, its role in hematopoietic granulopoiesis has not yet been addressed.

**Methods:** We analyzed the effect of hematopoietic specific Cre-lox deletion of XBP-1 on basophil, mast cell, eosinophil and neutrophil homeostasis by flow cytometry, microarray analyses, RT-qPCR and mixed bone marrow chimeras. Morphological studies were performed with transmission electron microscopy and Wright-Giemsa staining cytopun cell preparations.

**Results:** Targeted hematopoietic ablation of XBP-1 or its upstream activator IRE-1 $\alpha$  with Mx1-Cre or Vav1-Cre resulted in complete loss of mature eosinophils and dramatic decrease in Lin<sup>-</sup>Sca1<sup>+</sup>CD34<sup>+</sup>C-kit<sup>lo</sup>IL-5R $\alpha$ <sup>+</sup> eosinophil progenitors without altering basophil, neutrophil, or mast cell populations ( $p < 0.004$ ). This developmental defect could not be rescued *in vitro* in bone marrow cells cultured under conditions that drive eosinophilogenesis. Furthermore, mixed bone marrow chimera experiments demonstrated that the function of XBP-1 is cell intrinsic. Microarray and qPCR analyses of purified eosinophil progenitors demonstrated that proteostasis and endoplasmic reticulum stress response regulators were selectively downregulated in XBP-1-deficient cells, while previously described eosinophil developmental regulators such as GATA-1 and C/EBP $\alpha$  were unaffected. Cytospin analyses after enforced expression of the transcription factor GATA2 in purified granulocyte macrophage precursors, a procedure which has been shown to drive eosinophil development, revealed that XBP-1 deficient bone marrow cells are capable of initiating granule formation, but that these cells commit to the eosinophil lineage less frequently than their wild type counterparts and develop hypomorphic granules.

**Conclusions:** Our findings identify a novel role for the transcription factor XBP-1 in selectively regulating the development of the eosinophil lineage, but not other granulocyte lineages, and implicate the IRE-1 $\alpha$ /XBP-1 signaling axis as a potential therapeutic target for eosinophil-mediated diseases.



**GENETIC BASIS OF FAMILIAL EOSINOPHILIA**

Zeynep Kalender<sup>1</sup>, Senbagavalli Prakash<sup>2</sup>, Jian Du<sup>3</sup>, Kim De Keersmaecker<sup>1</sup>, Stein Aerts<sup>1</sup>, Thomas B. Nutman<sup>2</sup>, Steven J. Ackerman<sup>3</sup>, Jan Cools<sup>1</sup>, Amy D. Klion<sup>2</sup>

<sup>1</sup>Center for Human Genetics, K.U. Leuven, Belgium; <sup>2</sup>Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD <sup>3</sup>University of Illinois at Chicago, Chicago, IL

**Background:** We have previously described a 5-generation kindred with autosomal dominant transmission of marked eosinophilia (>1,500/mL). Whereas most affected family members remain asymptomatic with a normal life span, progression to hypereosinophilic syndrome has occurred in 3 family members. The gene responsible for this disorder has been mapped to a 18 cM portion of chromosome 5q31-33 (LOD 6.49), but selective sequencing of candidate genes was unsuccessful at identifying a causative abnormality. Preliminary studies revealed no evidence of an intrinsic abnormality of eosinophil development, activation or apoptosis.

**Methods:** Peripheral blood mononuclear cells (PBMC) were purified from affected and unaffected family members. RNA was isolated from 9 affected and 8 unaffected family members for microarray analysis (Affymetrix U133). Whole genome sequencing was performed on an Illumina HiSeq sequencer using DNA from 2 affected and 2 unaffected family members. Data was filtered to look for single nucleotide variations (SNVs), interstitial deletions, structural variations and copy number changes unique to affected family members in the mapped region of chromosome 5. Sanger sequencing was used to confirm the presence of candidate mutations.

**Results:** In order to explore the potential role for lymphocytes in driving the eosinophilia in affected family members, microarray analysis of PBMC RNA from affected and unaffected family members was performed. Comparison of expression profiles of genes in the mapped area of chromosome 5 revealed marked upregulation of IL-5 expression in PBMC from affected family members. This was confirmed by TaqMan analysis, which demonstrated significant upregulation of IL-5 mRNA in affected family members (mean 1/DCT = 0.11 vs. 0.04 in affected vs. unaffected family members, respectively;  $p=0.02$ ). Whole genome sequencing identified 9 novel SNVs in the mapped region of chromosome 5q31-33 from both affected family members, including two SNVs in the 5' UTR of the IL-5 gene. Sanger sequencing confirmed that the two SNVs were present in all 4 affected family members and none of 4 unaffected family members tested. In order to determine the functional implications of these SNVs on IL-5 mRNA expression, fragments corresponding to the region containing two candidate SNVs have been amplified from DNA from an affected and an unaffected family member to assess their effect on promoter activity in a luciferase reporter vector (pGL4) containing either a minimal viral promoter or the IL-5 promoter itself. Luciferase activity will be assessed in an HTLV1-transformed human Th2 cell line, that has been documented to constitutively produce high levels of IL-5 mRNA and protein.

**Conclusion:** Through a combination of genetic approaches, two promising candidate mutations have been identified in DNA from subjects with familial eosinophilia. Further studies to assess the ability of these mutations to regulate IL-5 promoter activity are currently underway.

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**ACTIVATED EOSINOPHILS RELEASE HIGH MOBILITY GROUP BOX 1 PROTEIN (HMGB1), A NOVEL IMMUNOREGULATORY MEDIATOR WITH AIRWAY INFLAMMATORY CELL-ACTIVATING CAPABILITIES.**

Christof Straub<sup>2</sup>, Rosario Maroto<sup>1</sup>, Bo Xu<sup>1</sup>, Konrad Pazdrak<sup>1,2</sup>, and Alexander Kurosky<sup>1,2</sup>.

<sup>1</sup>University of Texas Medical Branch NHLBI Proteomics Center at Galveston, <sup>2</sup>Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, Galveston, Texas, U.S.A.

**Background:** The high mobility group box 1 (HMGB1) protein is a proinflammatory cytokine that has recently become implicated in asthma pathogenesis; sputum and plasma HMGB1 levels have been positively correlated with asthma severity. Most asthma subtypes are marked by an influx of eosinophils into the airways and eosinophils are thus potential sources of HMGB1 in the airways. We investigated whether eosinophils can secrete HMGB1 and whether eosinophil-derived HMGB1 affects asthma-associated immune cells as well as resident airways cells.

**Methods:** The presence of HMGB1 in eosinophils and its Lys acetylation modification were determined using 2-dimensional gel electrophoresis and mass spectrometry. Eosinophil-secreted HMGB1 (sHMGB1) was purified through sequential cation-exchange and hydrophobic interaction chromatography steps. sHMGB1 was then characterized by SDS-PAGE and mass spectrometry. Post-translational modifications were analyzed using western blotting and LC-MS/MS. Conditioned culture medium containing sHMGB1-stimulated monocytes as well as bronchial epithelial cells was analyzed for the presence of cytokines and chemokines using a bead-based multiplex assay system. Neutrophil chemotaxis experiments were conducted using a transwell system (Corning).

**Results:** Eosinophils express HMGB1 primarily in the nucleus and stimulation with GM-CSF leads to HMGB1 acetylation and subcellular relocation to the cytoplasm. GM-CSF stimulation caused the secretion of HMGB1 in significant amounts (21.6 +/- 2.6 ng/mL medium; control 1.2 +/- 0.2 ng/mL). sHMGB1 was acetylated, in part, on Lys 90 and Lys114. We found that purified sHMGB1 elicited the release of IL-12, G-CSF, MCP-1, and TNF- $\alpha$  from monocytes at statistically significant levels when compared to sham treated monocytes. Primary bronchial epithelial cells released IL-10, IL-13, and VEGF in response to sHMGB1. In addition, we observed that sHMGB1 was a potent chemoattractant for neutrophils.

**Conclusions:** Our results demonstrate for the first time that eosinophils when activated by various cytokines can secrete the proinflammatory cytokine HMGB1. Furthermore, secreted HMGB1 causes the release of proinflammatory cytokines, from monocytes, e.g. IL-12, G-CSF, MCP-1 and TNF- $\alpha$ , and from bronchial epithelial cells, e.g. IL-10, IL-13, and VEGF. Thus, we propose that eosinophil-derived HMGB1 directly impacts asthma-associated cells and importantly plays a role in airway inflammation.

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**A ROLE FOR EOSINOPHILS IN PROMOTION OF B CELL PROLIFERATION**

Tina W. Wong<sup>1</sup>, Alfred D. Doyle<sup>2</sup>, James J. Lee<sup>2</sup>, Diane F. Jelinek<sup>1</sup>

<sup>1</sup>Department of Immunology, Mayo Clinic, Rochester, MN; <sup>2</sup>Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ

**Background:** The observation that NJ1638 (IL-5 transgenic) hypereosinophilic mice exhibit B cell lymphocytosis together with our recent demonstration of eosinophils' (Eos) ability to induce malignant human plasma cell proliferation prompted us to examine the contribution of Eos to normal B cell proliferation in both mice and humans.

**Methods:** **MOUSE:** To assess the etiology of the B cell lymphocytosis in NJ1638 mice (i.e., IL-5 overexpression vs. hypereosinophilia), we crossed NJ1638 mice with the Eos deficient mouse, PHIL. Peripheral blood (PB) differentials from each of the resulting genotypes (WT, NJ1638, PHIL, NJ1638/PHIL) were analyzed via flow cytometric methods.

**HUMAN:** Tonsil and spleen sections were subjected to H&E and immunofluorescence staining to determine the localization of Eos relative to germinal centers. To assess the ability of Eos to augment B cell proliferation, B cells were isolated from PB, tonsils, and spleens of normal donors and co-cultured with Eos ± additional stimulation. B cell proliferation was assessed by <sup>3</sup>H-thymidine-incorporation and the contact-dependency of the Eos-enhanced B cell proliferation was evaluated using transwell co-cultures. Immunoglobulin levels in culture supernatants were quantified using ELISA.

**Results:** **MOUSE:** Flow cytometric assessments of PB leukocytes from NJ1638 and NJ1638/PHIL mice revealed that the elevated B cell numbers in NJ1638 are dependent upon the presence of Eos (4,857 vs. 855 B cells/μl peripheral blood).

**HUMAN:** Histologic analysis demonstrated that Eos are found at the T cell-B cell border in secondary lymphoid structures. *In vitro* studies showed that, at day 3 of culture, B cell proliferation at baseline and upon CpG stimulation is enhanced 1.5-2 fold in the presence of Eos. Immunoglobulin levels in day 10 culture supernatants were also elevated more than 2-fold in the presence of Eos. Transwell cultures revealed that both contact-dependent and -independent mechanisms may contribute to the promotion of B cell proliferation by Eos. B cells isolated from PB, tonsils, and spleens all exhibited this proliferative response in coculture with Eos. Furthermore, the proliferation of CpG-stimulated naïve (CD19+CD27-) and memory (CD19+CD27+) subsets of PB B cells was equally augmented by Eos. Finally, we demonstrate that the ability of Eos to augment CpG-stimulated B cell proliferation can be achieved after only a transient exposure to Eos (during the first 24 hours of activation).

**Conclusions:** Hypereosinophilic (NJ1638) mice exhibit B cell lymphocytosis that can be ameliorated via the removal of Eos from these mice despite the continued constitutive expression of the IL-5 transgene. Human *in vitro* studies demonstrate that purified Eos can enhance B cell proliferation which may involve both contact-dependent and -independent mechanisms. The requirement of only a transient interaction between Eos and B cells to result in augmented B cell proliferation mimics the *in vivo* departure of B cells from Eos at the T-B border upon activation followed by germinal center formation.

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**EMRI: A NOVEL THERAPEUTIC TARGET FOR EOSINOPHILIC DISORDERS**

Fanny Legrand<sup>1</sup>, Olga Simakova<sup>2</sup>, Chyi-Chia Richard Lee<sup>3</sup>, Zengfang Wang<sup>3</sup>, Mark Raffeld<sup>3</sup>, Michelle A. Makiya<sup>1</sup>, Varghese Palath<sup>4</sup>, John Leung<sup>4,5</sup>, Mark Baer<sup>4</sup>, Nenad Tomasevic<sup>4,5</sup>, Geoffrey Yarranton<sup>4</sup>, Christopher Bebbington<sup>4,5</sup>, Irina Maric<sup>2</sup>, Amy D. Klion<sup>1</sup>

<sup>1</sup>Eosinophil Pathology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases;

<sup>2</sup>Department of Laboratory Medicine, Clinical Center

<sup>3</sup>Laboratory of Pathology, National Cancer Institute;

<sup>4</sup>KaloBios Pharmaceuticals Inc.

<sup>5</sup>current address: Allakos Inc.

**Background:** Eosinophils are implicated in the pathogenesis of a wide variety of disorders, including asthma, parasitic helminth infection, and hypereosinophilic syndromes (HES). Although glucocorticoids are the first line therapy for many of these disorders, long-term toxicity and/or resistance are common. Despite clinical trials demonstrating the safety and efficacy of novel monoclonal antibodies targeting eosinophils in asthma and HES, none has shown 100% efficacy in reducing tissue eosinophils or clinical symptoms. Human epidermal growth factor (EGF)-like module containing mucin-like hormone receptor (EMR1) is a surface receptor of unknown function. Unlike its murine analog, F4/80, which is expressed on monocytes and macrophages, human EMR1 has been reported to be expressed only on eosinophils.

**Methods:** To explore the potential of EMR1 as a target for the treatment of eosinophilia, the pattern of EMR1 expression was assessed in blood and tissue specimens from eosinophilic subjects and normal controls, as well as in various cell lines and CD34+ cells differentiated *in vitro* to promote eosinophils development. The ability of afucosylated anti-EMR1 IgG1 to target eosinophils was evaluated *in vitro* by NK killing assay and *in vivo* in cynomolgus monkeys.

**Results:** Flow cytometric and real-time PCR analysis of blood and bone marrow cells from normal (n=16) and eosinophilic donors (n=21), as well as CD34+ cells cultured *in vitro*, confirmed that human EMR1 is expressed exclusively on mature eosinophils. EMR1 expression was also detected by immunostaining on eosinophils in skin and nasal polyp biopsies. Whereas EMR1 was highly expressed on blood eosinophils from all donors tested (geometric mean DMFI=3697, range=943-14570), surface expression was negatively correlated with absolute eosinophil count (AEC) ( $r = -0.46$ ,  $P < .001$ ), suggesting modulation of EMR1 *in vivo*. Incubation of purified eosinophils *in vitro* with interleukin-5 revealed a similar pattern of EMR1 regulation. Plasma levels of soluble EMR1 were positively correlated with AEC ( $r = 0.69$ ,  $P < 0.001$ ), consistent with receptor shedding. Afucosylated anti-EMR1 antibody enhanced NK killing of eosinophils *in vitro* in both normal and eosinophilic donors (geometric mean Annexin V+ cells increased from 15.1 to 62.3%; n=6;  $P < 0.05$ ). Furthermore, afucosylated anti-EMR1 (1 mg or 5 mg iv) was well-tolerated and induced a rapid (<8 hrs) and sustained (> 1 month) decrease in AEC in 4 cynomolgus monkeys.

**Conclusions:** These data suggest that EMR1 is highly and selectively expressed on blood and tissue eosinophils, and that targeting eosinophils using afucosylated anti-EMR1 may be a promising strategy for the treatment for eosinophilic disorders.

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**EOSINOPHIL DEGRANULATION IS REGULATED BY VAMP-7-MEDIATED EXOCYTOSIS, *IN VIVO***

Lian Willetts<sup>1,2</sup>, Elizabeth A. Jacobsen<sup>2</sup>, Rachel M. Condjella<sup>2</sup>, Katie Zellner<sup>2</sup>, Sergei I. Ochkur<sup>2</sup>, Hui Lou<sup>2</sup>, Will E. LeSuer<sup>2</sup>, John D. Kim<sup>1,2</sup>, Cheryl A. Protheroe<sup>2</sup>, Ralph S. Pero<sup>2</sup>, Nancy A. Lee<sup>2</sup>, Paige Lacy<sup>1</sup>, James J. Lee<sup>2</sup>, Redwan Moqbel<sup>3</sup>.

<sup>1</sup> Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB, Canada

<sup>2</sup> Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ, USA

<sup>3</sup> Department of Immunology, University of Manitoba, Winnipeg, MB, Canada

**Background:** Eosinophil degranulation is a potentially critical process in allergic inflammation, although direct evidence for its role in the pathogenesis of inflammation is lacking. Degranulation from eosinophils appears to occur through regulated exocytosis or by necrosis (cytolysis). Regulated exocytosis in eosinophils is dependent on vesicle associated membrane protein (VAMP), a prominent component of the universal fusion core machinery (soluble NSF attachment protein receptor (SNARE) complex). We have previously demonstrated a major role for VAMP-7 in human eosinophil exocytosis (Logan et al. (2006) Allergy), and have hypothesized that VAMP-7 is critical for mouse eosinophil degranulation and that gene deletion of VAMP-7 in mouse eosinophils results in ablated exocytosis of secondary granule proteins *ex vivo* and *in vivo*.

**Objectives:** To generate and characterize a mouse model of eosinophil-specific VAMP-7 gene deletion, and evaluate the role of VAMP-7 in regulated eosinophil exocytosis in the context of inflammatory models of human diseases.

**Methods:** We crossed an eosinophil-specific Cre recombinase-expressing strain of mice (eoCRE) with animals that have a modified VAMP-7 locus flanked by two Lox P Cre recognition sequences ("floxed VAMP-7" mice) to achieve VAMP-7 gene deficiency exclusively in eosinophils. Eosinophils were tested for release (i.e., degranulation) of eosinophil peroxidase (EPX), major basic protein (MBP), and eosinophil-associated ribonucleases (Ears) using multiple assays: (i) *in vitro* stimulation with platelet-activating factor (PAF) with or without Ca<sup>2+</sup> ionophore; and (ii) *ex vivo* degranulation following adoptive transfer of eosinophils into the airways of compound interleukin-5 (IL-5)/human eotaxin-2 (hE2) double transgenic mice that were also deficient in EPX gene expression (IL-5/hE2/EPX<sup>-/-</sup>).

**Results and Conclusions:** eoCRE mice were crossed with floxed VAMP-7 animals to generate eoCRE/V7 mice. eoCRE/V7 mice displayed a hematopoietic profile identical to wild-type animals. However, eoCRE/V7 mice exhibited gene deletion in >95% eosinophils, with no evidence of deletion in other cell types. Following *in vitro* stimulation of VAMP-7-deficient eosinophils with PAF and ionomycin, degranulation was significantly reduced relative to wild-type as assessed by EPX sandwich ELISA and single dimensional immunoblot assays for MBP and Ears. This inhibition of degranulation was confirmed *ex vivo* following adoptive transfer of eoCRE/V7/EPX<sup>+/+</sup> eosinophils into the airways of IL-5/hE2/EPX<sup>-/-</sup> mice. These data suggest that VAMP-7-mediated granule exocytosis is a key component of eosinophil degranulation occurring in this mouse model.

**Future Directions:** Significant eosinophil degranulation in mice with eosinophil-specific VAMP-7-deficiency provides us with a unique opportunity to test the importance of SNARE-mediated granule protein release in animal models of eosinophilic diseases. In particular, our current studies are examining the role(s) of VAMP-7-mediated eosinophil effector functions in pathologies associated with allergen provocation of the lung (asthma models) and the skin (atopic dermatitis/allergic contact dermatitis models).

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## A CRITICAL ROLE FOR SMALL INTESTINAL EOSINOPHILS IN INITIATING FOOD ALLERGIC TH2 IMMUNITY

Derek K. Chu<sup>1</sup>, Tina D. Walker<sup>1</sup>, Susanna Goncharova<sup>1</sup>, Alba Llop-Guevara<sup>1†</sup>, Nicole G. Barra<sup>1</sup>, Jennifer D. Bassett<sup>1</sup>, Rodrigo Jimenez-Saiz<sup>1</sup>, Juliana L. Xie<sup>1</sup>, Jonas S. Erjefält<sup>5,6</sup>, Ramzi Fattouh<sup>3</sup>, Karen L. Mossman<sup>1</sup>, Roland Kolbeck<sup>4</sup>, Alison A. Humbles<sup>4</sup>, Susan Wasserman<sup>2</sup>, Kathy D. McCoy<sup>7</sup>, Manel Jordana<sup>1</sup>

<sup>1</sup>McMaster Immunology Research Centre (MIRC), Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

<sup>2</sup>Department of Medicine, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

<sup>3</sup>Cell Biology Program, Hepatology, and Nutrition, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

<sup>4</sup>Department of Respiratory, Inflammation and Autoimmunity, MedImmune LLC, Gaithersburg, MA, 20878, USA.

<sup>5</sup>Department of Experimental Medical Science, Lund University, Lund, SE-22184, Sweden.

<sup>6</sup>Department of Respiratory Medicine and Allergology, Lund University Hospital, Lund, SE-22185, Sweden.

<sup>7</sup>Maurice Müller Laboratories, Universitätsklinik für Viszerale Chirurgie und Medizin (UVCM), University of Bern, 3008 Bern, Switzerland.

**Background:** Eosinophils are classically considered as terminal effector leukocytes under the direction of adaptive Th2 immunity in anti-parasitic and allergic immune responses. However, eosinophils natively inhabit the small intestine, where food is digested and absorbed. A functional role for them there has remained elusive for over 100 years.

**Methods:** We characterized intestinal eosinophils by flow cytometry and investigated their contribution to intestinal and adaptive immunity. Wild-type (WT) and eosinophil-deficient mice were compared in their ability to generate Peyer's patch tertiary lymphoid tissues, IgA secretion, and robustness of oral tolerance versus food allergy induction.

**Results:** Eosinophils were most prevalent within the small intestine (SI), ranging from ~10-20% of all cells. In contrast, the large intestine (LI) contained <1-10% eosinophils. Although both SI and LI eosinophils were Siglec-F<sup>+</sup>, CD11b<sup>+</sup>, CD44<sup>+</sup>, F4/80<sup>int</sup>, CD11c<sup>int to neg</sup>, and ~30% Ly6G<sup>+</sup>, SI and LI Eosinophils also had distinct cell surface phenotypes. SI Eosinophils expressed ST2 (IL-33 receptor), CD69, and Ly6C, whereas LI Eosinophils did not. Functionally, WT and eosinophil-deficient mice did not display any differences in tertiary lymphoid tissue organogenesis, macronutrient uptake, or IgA secretion. Notably, whereas both mice can mount robust IgE responses, both strains also mounted robust oral tolerance induction. In contrast, induction of food allergy was markedly impaired, including IgG1 and IgE induction, clinical anaphylaxis, inflammatory responses, and Th2 cytokine production. However, if food allergy is induced through per rectum immunization, robust Th2 responses are induced in both WT and eosinophil-deficient mice.

**Conclusions:** Different compartments of the intestine harbour different numbers and phenotypes of eosinophils, suggesting potentially different functional capacities. In contrast to the conventional notion of eosinophils as terminal effector leukocytes, we show here that small intestinal eosinophils instead play a critical role in initiating food allergic adaptive immunity.

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**DETERMINATION OF THE MOLECULAR PHENOTYPE OF ESOPHAGEAL MUCOSAL INFLAMMATION IN CHILDREN WITH EOSINOPHILIC ESOPHAGITIS USING A 1-HOUR ESOPHAGEAL STRING TEST**

Steven J. Ackerman,<sup>1</sup> Amir F. Kagalwalla,<sup>2</sup> Preeth Alumkal,<sup>1</sup> Jian Du,<sup>1</sup> Katie Amsden,<sup>2</sup> Sabina Mughal,<sup>2</sup> Wendy Moore,<sup>3</sup> Lindsay Hosford,<sup>3</sup> Rachel Harris,<sup>3</sup> Ha Na Choe,<sup>3</sup> Sophie Fillon,<sup>3</sup> Joanne C. Masterson,<sup>3</sup> Kelly Capocelli,<sup>4</sup> Hector Melin-Aldana,<sup>5</sup> Brian T. Maybruck,<sup>1</sup> Sergei Ochkur,<sup>6</sup> James J. Lee,<sup>6</sup> Zhaoxing Pan<sup>7</sup> and Glenn T. Furuta<sup>3</sup>

<sup>1</sup>Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, <sup>2</sup>Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, IL, <sup>3</sup>Gastrointestinal Eosinophilic Diseases Program, Section of Pediatric Gastroenterology, Hepatology and Nutrition and Mucosal Inflammation Program, University of Colorado School of Medicine, Aurora, CO; Department of Pediatrics, National Jewish Health Denver CO; Digestive Health Institute and, Children's Hospital Colorado, Aurora, CO, <sup>4</sup>Department of Pathology, Children's Hospital Colorado, University of Colorado Denver, Chicago, CO, <sup>5</sup>Department of Pathology, Ann & Robert H. Lurie Children's Hospital, Northwestern University Feinberg School of Medicine, Chicago, IL and <sup>6</sup>Department of Biochemistry and Molecular Biology, S.C. Johnson Medical Research Center, Mayo Clinic Arizona, Scottsdale, AZ and <sup>7</sup>Departments of Pediatrics and Biostatistics and Informatics, University of Colorado Denver, and Children's Hospital Research Institute, Aurora, CO, USA.

**Background:** Eosinophilic Esophagitis (EoE) is a chronic disease characterized by eosinophil-predominant esophageal inflammation for which endoscopy with biopsy is currently the standard method to assess mucosal inflammation and follow resolution with treatment. However, we recently reported that eosinophil secreted granule proteins (ESGPs) can be captured and assayed from esophageal secretions, and used to distinguish active EoE from EoE in remission, GERD and normal using a minimally invasive Esophageal String Test (EST) after leaving it in situ for 12-hours (1).

**Aims:** Our objectives were to determine whether the EST could capture esophageal luminal biomarkers associated with esophageal eosinophilia in a shorter 1-hour sampling period and differentiate the activity of mucosal inflammation in children with EoE.

**Methods:** Four hours before endoscopy with biopsy was performed, an EST was swallowed. After a 1-hour sampling period, the EST was removed and endoscopy was performed 3 hours later. ESGPs [major basic protein-1 (MBP1) and eosinophil peroxidase (EPX)], and Th2 cytokines (IL-4, IL-5, IL-13) and chemokines (Eotaxin-2, -3) were measured by ELISA in luminal effluents (secretions, cells) eluted from the ESTs and in extracts of esophageal biopsies obtained at the time of endoscopy (1).

**Results:** Samples from 21 children (ages 9-18) with active EoE (n=5; 19-120 eos/HPF), treated EoE in remission (n=5; 0-9 eos/HPF), GERD (n=3; 0-2 eos/HPF) and normal esophagus (n=8; 0-1 eos/HPF) were analyzed. EST measurements of MBP1 significantly differentiated between children with active EoE (8.07±3.6mg/ml), EoE in remission (0.59±0.17mg/ml), GERD (0.36±0.01mg/ml) and normal esophagus (0.04±0.02mg/ml) (all p<0.01), while EPX distinguished between active EoE (0.97±0.48mg/ml), GERD (0.05±0.02mg/ml) and normal esophagus (0.07±0.02mg/ml) (all p<0.01). EST measurements of eotaxin-2 differentiated between active EoE (756±356pg/ml) and treated EoE in remission (82±24pg/ml) (p<0.05) and normal (99.7±15pg/ml, p=0.052), while Eotaxin-3 differentiated between active EoE (442±200pg/ml) and normal esophagus (2.5±2.5pg/ml) (p<0.05). Th2 cytokines (IL-4, IL-5, IL-13) were not detectable in 1-hour EST samples (all below sensitivity of the ELISAs). EST MBP1 (r=0.972, p< 0.001) and EPX (r=0.751, p<0.001) biomarker levels correlated significantly with the peak number of eosinophils in biopsies, as did EST levels of Eotaxin-2 (r= 0.917, p<0.001) and Eotaxin-3 (r=0.917, p<0.001).

**Conclusions:** Eosinophil-associated biomarkers that are captured by the EST in a clinically relevant time frame reflect mucosal inflammation and disease activity in EoE. The EST is a novel, time efficient, minimally invasive device for measuring esophageal eosinophilic inflammation in children with EoE.

**Reference:** (1) Furuta GT et al and Ackerman SJ. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. *Gut*. 2012 Aug 15 [Epub ahead of print] PMID: 22895393.

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## PURINERGIC RECEPTOR P2Y12 FUNCTIONAL ROLES IN HUMAN ISOLATED EOSINOPHILS AND IN THE SCHISTOSOMAL HOST RESPONSE

Valdirene S. Muniz<sup>1</sup>, Renata B. Reis<sup>1</sup>, Hilton A. Mata-Santos<sup>2</sup>, Alexandre S. Pyrrho<sup>2</sup>, Peter F. Weller<sup>3</sup>, Rodrigo T. Figueiredo<sup>4</sup>, Josiane S. Neves<sup>1</sup>

<sup>1</sup>Institute of Biomedical Sciences, <sup>2</sup>Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil, <sup>3</sup>Division of Allergy and Inflammation, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, USA, <sup>4</sup>Institute of Biomedical Sciences/Unit of Xerem, Rio de Janeiro, RJ, Brazil.

**Background:** Identification of new target molecules through which eosinophils activate and secrete their stored proteins may be highly significant for our understanding about the pathophysiology of host immune responses to parasites and allergic inflammation, as well as reveal new therapeutic targets for the control of the eosinophilic disorders. We have recently reported the expression of the purinergic P2Y12 receptor (P2Y12R) in human eosinophils (JACI 125:477-482, 2010). However its functional role in this cell type and involvement in eosinophilic inflammation are still unknown. In this work we investigated the expression and the functional roles of the P2Y12R in isolated human eosinophils and in a murine model of eosinophilic inflammation (*Schistosoma mansoni*).

**Methods:** We isolated eosinophils from blood of healthy donors by negative immunomagnetic selection. P2Y12R protein expression, eosinophil apoptosis and chemotaxis were evaluated by flow cytometry. Eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) measurements were assessed by colorimetric assays. *In vivo*, C57Bl/6 mice were infected with 50 cercariae of the BH strain of *S. mansoni* by the cutaneous route (protocol licence CEUA/UFRJ DFBCICB043). The animals were treated with a P2Y12R antagonist, clopidogrel (500µg/mL), via the drinking water three days before and throughout the infection period (55 days). Histopathological and biochemical analyses were performed in the liver to evaluate the areas of inflammatory granulomatous infiltration, collagen deposition and IL-13 production. Blood eosinophilia and eosinophil count in the bone marrow were assessed after blood smears and cytopsin analyses, respectively.

**Results:** Functionally, we found that ADP induced isolated human eosinophils to secrete cationic proteins, being the EPO secretion clearly dependent on the P2Y12R activation. In contrast ADP did not interfere with human eosinophil apoptosis or promoted eosinophil direct chemotaxis. In a relevant murine model of eosinophilic inflammation (*S. mansoni*) we found that the P2Y12R blockage reduced the area of the hepatic granuloma (not treated:  $5.47 \pm 1.8$  versus treated  $4.2 \pm 1.6$  ( $\times 10^4$   $\mu\text{m}^2/\text{granuloma}$ ), means  $\pm$  EPM, N=5), promoted a suggestive reduction of the eosinophilic granuloma infiltration, as well as inhibited collagen deposition (not treated:  $880 \pm 7$  versus treated  $690 \pm 6$  ( $\mu\text{g}$  hydroxyproline/g liver), means  $\pm$  EPM, N=5) and IL-13 production (reduction of 22%, N=5) in the liver without altering the parasite oviposition. Furthermore, the P2Y12R inhibition promoted blood eosinophilia (2-fold increase, N=5), whereas decreased the eosinophil count in the bone marrow (60% reduction, N=5).

**Conclusion:** Taken together our results suggest that the P2Y12R has an important role in the eosinophil activation and cationic protein secretion, as well as in the establishment of the eosinophilic inflammation.

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**SCHISTOSOMAL-DERIVED LYSOPHOSPHATIDYLCHOLINE TRIGGERS IL-13 AND TGF $\beta$  SECRETION BY EOSINOPHILS THROUGH 15-LIPOXYGENASE DEPENDENT MECHANISMS**

Kelly G. Magalhães<sup>1,3,4</sup>, Tatiana Luna<sup>2</sup>, Christianne Bandeira-Melo<sup>2</sup>, Peter F. Weller<sup>4</sup> and Patricia T. Bozza<sup>1</sup>

<sup>1</sup>Lab. Imunofarmacologia, Instituto Oswaldo Cruz, FIOCRUZ, Brazil; <sup>2</sup> Lab. Inflamação UFRJ-Brazil; <sup>3</sup>Lab. Imunologia e Inflamação, Universidade de Brasília, Brazil, <sup>4</sup>Lab. Allergy and Inflammation, Harvard Medical School, USA.

**Background:** Remarkable accumulation of tissue eosinophils and fibrosis are characteristic features of *Schistosoma mansoni* infection. Recently, we have demonstrated roles for schistosomal-derived lipids in eosinophil recruitment, granuloma formation and host-pathogen interactions in a murine model of *S. mansoni* infection [Magalhaes et al., J Infectious Dis 2010, 202:1369]. However, the effects of schistosomal-derived lipids in the direct activation of eosinophils are not clear.

**Objectives:** Here we investigate the effects of schistosomal-derived lipids in the immunomodulatory functions of eosinophils.

**Methods:** Purified human eosinophils or Balb/c bone marrow derived eosinophils were incubated for 1 h at 37°C with adult worm-derived schistosomal lipid extract and schistosomal-derived lysophosphatidylcholine (LPC) or arachidonic acid (AA). Pre-treatments were performed for 30 min with neutralizing antibody against TLR2 or 15-lipoxygenase inhibitor-1. Quantification of cytokines, LTC<sub>4</sub> and EXC<sub>4</sub> (14,15 LTC<sub>4</sub>) levels was carried out by ELISA, LUMINEX and EIA kits. Immunodetection of intracellular EXC<sub>4</sub> was determined by EicosaCell assay.

**Results:** Total lipid extract from *S. mansoni* or isolated schistosomal-derived LPC activates human and murine eosinophils eliciting synthesis of LTC<sub>4</sub> and EXC<sub>4</sub>. Interestingly schistosomal-derived LPC lead to increased expression of 15-LO and an increased ratio of EXC<sub>4</sub> over LTC<sub>4</sub> production when compared to AA. Schistosomal-derived LPC significantly induced eosinophils to secrete IL-13 and TGF $\beta$ . Pre-treatment with anti-TLR2 inhibited schistosomal derived-LPC-induced EXC<sub>4</sub> and IL-13 by eosinophils, indicating that TLR2 mediates schistosomal lipids-driven eosinophil activation. Moreover, IL-13 and TGF $\beta$  secretion by eosinophils were abrogated by inhibition of 15-LO.

**Conclusions:** Taken together, our results showed that schistosomal lipids are capable of direct activation of human and murine eosinophils to release EXC<sub>4</sub> and the profibrotic cytokines IL-13 and TGF $\beta$  through mechanisms dependent of TLR-2 and 15-LO. Thus, by producing profibrotic mediators our findings suggest immunomodulatory roles for eosinophils in the pathogenesis of schistosomiasis-induced liver fibrosis.

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**NOVEL IL-9-PRODUCING INNATE HELPER CELLS PROMOTE IGE-MEDIATED EXPERIMENTAL FOOD ALLERGY**

Chun-Yu Chen<sup>1</sup>, Jee-Boong Lee<sup>1</sup>, Bo Liu<sup>1</sup>, Karen Burwinkel<sup>1</sup>, Fred D. Finkelman<sup>2,3,4</sup>, Simon P. Hogan<sup>1</sup>, Yui-Hsi Wang<sup>1</sup>

<sup>1</sup>Division of Allergy and Immunology, <sup>2</sup>Division of Immunobiology, Cincinnati Children's Hospital Medical Center, <sup>3</sup>Department of Medicine, Cincinnati Veterans Affairs Medical Center, <sup>4</sup>Division of Immunology, University of Cincinnati College of Medicine

**Background:** The manifestation of IgE-mediated experimental food allergy requires intestinal anaphylaxis driven by interleukin (IL)-9. However, the primary cellular source of IL-9 and mechanisms underlying intestinal anaphylaxis remain unclear.

**Methods:** Mice were sensitized twice within a two-week interval by intraperitoneal injection with 100 mg OVA and 1 mg alum before orally gavaged with 50 mg of OVA in 250 ml saline six times within two weeks and subsequently examined for food allergy symptoms. Intestinal lamina propria (LP) cells isolated from mice with active systemic anaphylaxis were characterized by multicolor immunofluorescence staining for surface markers and intracellular cytokines. Serum samples were analyzed using ELISA kits of OVA-specific IgE, MCPT-1, and OVA-specific IgG1. For intestinal histological analyses, duodenal tissue was fixed and stained with Leder stain for chloroacetate esterase (CAE) activity in intestinal MCs or periodic acid-Schiff (PAS) for mucins in goblet cells.

**Results:** We have identified the multifunctional IL-9-producing innate helper cells (IHC9) that promote intestinal TH2 inflammation and mastocytosis, thus driving the development of IgE-mediated experimental food allergy. Phenotypic analysis reveals that IHC9 lack lineage markers and express ST2 (the IL-33 receptor), FcεR, and c-kit. IHC9 secrete prodigious amounts of IL-9 (~2.0pg/mL per cell) as well as other TH2 cytokines, including IL-4 and IL-13, in lesser amounts, but do not secrete IFN-γ. IHC9 secrete mast cell protease-1 (MCPT-1) in response to antigen/IgE complex crosslinking and possess molecular signatures of mast cell lineage. Cytology showed that IHC9 were small in size and contained large nuclei, scanty cytoplasm, and few metachromatic granules. Electron microscopy revealed that IHC9 displayed an innate helper cell-like morphology. Repeated intragastric antigen challenge induces accumulation of intestinal IHC9, which correlates positively with symptoms and susceptibility to develop IgE-mediated experimental food allergy. The induction of IHC9 requires T cells and IL-4R, but not IL-9 or IL-9R signaling. The failure of irradiated mice reconstituted with STAT6-deficient bone marrow to generate IHC9 in response to challenge was accompanied by reduced serum MCPT-1, intestinal mastocytosis and goblet cell hyperplasia and loss of allergic diarrhea. Furthermore, inhibition of food allergy symptoms in mice ablated of IHC9 by anti-FcεR1a antibody treatment could be reversed by adoptive transfer of IHC9.

**Conclusion:** IHC9 represent the professional IL-9 producer and play a pivotal role in the development of IgE-mediated food allergy.

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**EOSINOPHILS ARE ESSENTIAL FOR PROGRESSION OF AUTOIMMUNE MYOCARDITIS TO DILATED CARDIOMYOPATHY**

Nicola L. Diny<sup>1,2</sup>, G. Christian Baldeviano<sup>1,2,3</sup>, Jobert G. Barin<sup>1</sup>, Monica Talor<sup>1</sup>, Jillian Legault<sup>1,2</sup>, Su F. Ong<sup>1,2</sup>, Lei Wu<sup>1,2</sup>, Noel R. Rose<sup>1,2</sup>, Daniela Cihakova<sup>1</sup>

<sup>1</sup>Department of Pathology, the Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

<sup>2</sup>W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

current address: Department of Parasitology, U.S. Naval Medical Research Unit Six (NAMRU-6), Lima, Peru

Eosinophilic myocarditis is a rare disease associated with cardiac interstitial infiltration of eosinophils. Although it can occur in the context of hypereosinophilic syndrome (HES), the most severe form of eosinophilic myocarditis, necrotizing eosinophilic myocarditis, is unrelated to systemic hypereosinophilia. It is characterized by extensive cardiomyocyte necrosis and carries a dire prognosis. The purpose of our study was to assess the pathogenic role of eosinophils in myocarditis and subsequent dilated cardiomyopathy (DCM). We established a novel model of eosinophilic myocarditis using BALB/c mice that overexpress IL-5 under the CD3 promoter (IL-5<sup>CD3</sup>). Naïve IL-5<sup>CD3</sup> mice showed marked eosinophilia, modeling HES, but did not develop spontaneous myocarditis even after mock immunization with adjuvant. In contrast, IL-5<sup>CD3</sup> mice developed severe myocarditis when immunized with a myocarditogenic peptide in adjuvant to induce experimental autoimmune myocarditis (EAM). Eosinophils accounted for over 50% of heart infiltrating cells, compared to only 1-3% in wildtype (WT) BALB/c mice with EAM, suggesting that injury in the heart is necessary for localization of circulating eosinophils. Immunized IL-5<sup>CD3</sup> mice also showed exacerbated DCM characterized by heart failure and pronounced cardiac remodeling and fibrosis. In patients, eosinophilic myocarditis can also occur in the absence of HES. We therefore addressed the role of eosinophils in a non-hypereosinophilic context. Myocarditis severity in eosinophil-deficient  $\Delta$ dblGATA1 mice was comparable to WT mice. However,  $\Delta$ dblGATA1 mice were completely protected from the development of DCM. Thus, eosinophils are necessary for the development of DCM. Next, we investigated the role of IL-5, a key cytokine for eosinophil expansion and survival, during EAM. IL5<sup>-/-</sup> mice showed no difference in inflammation in the early, acute phase (day 21) or in the later, chronic phase (day 36) of disease nor did they show differences in the ejection fraction compared to WT mice. Hence, IL5 is not required for the development of EAM or progression to DCM. Eotaxin signaling through its receptor, CCR3, was shown to be important for eosinophil trafficking to the lungs in asthma models. To determine whether this pathway also recruits eosinophils to the heart during myocarditis, we compared CCR3<sup>-/-</sup> and WT mice at day 21 post immunization. Myocarditis severity was comparable between CCR3<sup>-/-</sup> and WT mice. Nevertheless, CCR3<sup>-/-</sup> mice were largely protected from developing DCM on day 45 post immunization. In conclusion, eosinophils are not essential for development of myocarditis but are able to induce a severe myocarditis phenotype. Massive eosinophil infiltration in the heart leads to severe DCM. In addition, eosinophils are required for the development of DCM as eosinophil-deficient mice are protected from this chronic sequela of myocarditis.

**THE ROLE OF TYPE 2 INNATE LYMPHOID CELLS AND IL33 IN PERSISTENCE OF ASTHMA**

Christina Christianson, Chaoyu Irvin, Magdalena M. Gorska and Rafeul Alam

National Jewish Health and University of Colorado Denver, Denver, CO, USA

**Background:** We have previously reported the development of mouse model of chronic asthma by repetitive airway exposure to a combination of three allergens—dust mites, ragweed and *Aspergillus*. In this model the typical features of asthma (eosinophilic inflammation, airway hyperreactivity and remodeling) persist longer than 3 months after the cessation of the allergen exposure. In this study we compared the role of innate versus adaptive immune system and examined the importance of IL33 in sustenance of the typical features of asthma in the absence of the allergens.

**Methods:** Chronic asthma is induced through twice a week intranasal exposure to a combination of allergens (*D. Farinae*, ragweed, and *aspergillus*: DRA) for six weeks. Immune ablation was accomplished by lethal irradiation three weeks after the cessation of allergen exposure. Irradiated mice underwent transplantation of bone marrow from naïve mice. Airway hyperreactivity (airway resistance to inhaled methacholine by Flexivent) and immunohistological features were measured 2, 4 and 6 weeks after irradiation. Microarray of the lung tissue was performed using the One Array chips. Innate lymphoid cells (ILC) were identified in lung digest cells by flow cytometry after gating for live CD45+ cells.

**Results:** Immune and hematopoietic ablation was complete as mice that did not receive bone marrow transplantation died. Mice from the chronic asthma model maintained the typical features of asthma—airway hyperreactivity and airway remodeling at all observation points (week 2, 4 & 6) following immune ablation. They also mounted *de novo* inflammation that intensified beginning from week 4. The severity of the foregoing features was, nonetheless, milder than that in non-irradiated control chronic asthma mice. A microarray analysis of the lung tissue showed a significant increase in IL7, IL33 and IL7R from the chronic asthma model as compared to an acute asthma model and saline control. The heightened expression of IL33 persisted following immune ablation. The cytokine profile favors the development of ILC2. Indeed, we observed in this model a two-fold increase in the number of lung ILC2, which were characterized by lin-CD25+Sca1+c-kit+ST2+ and expression of IL5 and IL13 but not IL17. An anti-IL33 antibody abolished ILC2 in the lung, and inhibited airway hyperreactivity and inflammation that persisted after immune ablation. Administration of IL33 intranasally in naïve mice induced sustained features of asthma lasting longer than 2 weeks. This was associated with an increase in ILC2 in the lungs and auto-induction of IL33 mostly in surfactant protein-C (SPC)+ type 2 epithelial cells. The persistence of asthma following immune ablation was associated with sustained activation of ERK1/2 and AKT in airway epithelial cells, which was mimicked by IL33 administration in naïve mice.

**Conclusions:** We speculate that persistence of asthma is associated with the establishment of a self-sustained mechanism of generation of IL33 perhaps involving ERK1/2 and AKT signaling. Sustained generation of IL33 allows increased differentiation of ILC2. Together, they are able to maintain chronic features of asthma after ablation of the adaptive immune system.

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**USE OF 111-INDIUM-LABELLED AUTOLOGOUS EOSINOPHILS TO ESTABLISH *IN VIVO* KINETICS OF HUMAN EOSINOPHILS**

Neda Farahi<sup>1</sup>, Nanak R. Singh<sup>1</sup>, Sarah Heard<sup>2</sup>, Chrystalla Loutsios<sup>1</sup>, Charlotte Summers<sup>1</sup>, Chandra K. Solanki<sup>2</sup>, Kottekkattu K. Balan<sup>2</sup>, A. Michael Peters<sup>3</sup>, Alison M. Condliffe<sup>1</sup> and Edwin R. Chilvers<sup>1</sup>

<sup>1</sup>Respiratory Medicine Division, Department of Medicine, University of Cambridge, Cambridge, UK;

<sup>2</sup>Nuclear Medicine, Addenbrooke's Hospital, Cambridge, UK;

<sup>3</sup>Clinical Sciences Imaging Centre, Brighton and Sussex Medical School, Brighton, UK.

**Background:** Eosinophils are major cellular effectors of allergic inflammation and represent an important therapeutic target in asthma. While much is understood about the generation and activation of eosinophils, little is known about their intravascular kinetics and physiological fate. The purpose of this study was to image sites of eosinophil distribution and measure eosinophil kinetics in healthy individuals using autologous 111-Indium-labelled eosinophils.

**Methods:** To determine 'gold standard' kinetics of minimally manipulated eosinophils, mixed leukocytes were isolated from the blood of healthy volunteers, labelled with 111-Indium-tropolonate and re-injected. Blood was sampled 0.75-72 h post-injection. Neutrophils and eosinophils were isolated in parallel, and cell-associated radioactivity was measured. Using minimally manipulated granulocytes we found that the 45 min neutrophil recovery was  $57 \pm 10\%$  (n=7) and the intravascular lifespan was  $10.3 \pm 0.1$  h, in agreement with previous studies. By contrast, the 45 min recovery of eosinophils was  $15 \pm 2\%$  (n=7) and eosinophil lifespan was  $25.2 \pm 3.8$  h. To image sites of eosinophil margination/uptake eosinophils were purified using plasma-Percoll gradients and anti-CD16 immunomagnetic beads, labelled with 111-Indium-tropolonate and re-injected. The distribution of eosinophils was recorded by gamma camera dynamic imaging (0-40 min) followed by static imaging up to 72 h.

**Results:** Gamma camera imaging studies using purified eosinophils demonstrated initial retention in the lungs, with early re-distribution to the liver and spleen, and evidence of re-circulation from a hepatic pool (n=6). Simultaneous blood sampling showed that the 45 min recovery and intravascular lifespan of purified labelled eosinophils were  $15 \pm 3\%$  and  $30 \pm 2.7$  h, respectively, comparable to minimally manipulated cells.

**Conclusions:** This work provides the first *in vivo* measurements of eosinophil kinetics in healthy volunteers and shows that 111-Indium-labelled-eosinophils can be used to monitor the fate of eosinophils non-invasively.

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**ACTIVATED EOSINOPHILS PROTECT AGAINST A LETHAL RESPIRATORY VIRUS INFECTION**

Caroline M. Percopo<sup>1</sup>, Kimberly D. Dyer<sup>1</sup>, Sergei I. Ochkur<sup>2</sup>, Nancy A. Lee<sup>2</sup>, Joseph B. Domachowski<sup>3</sup>, James J. Lee<sup>2</sup> and Helene F. Rosenberg<sup>1</sup>

<sup>1</sup>Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA and <sup>2</sup>Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259 USA and <sup>3</sup>Department of Pediatrics, State University of New York, Upstate Medical University, 750 East Adams Street, Syracuse, New York, 13210 USA

**Background:** Asthma is an inflammatory disease of the airways that leads to reversible airflow obstruction, bronchospasm and pulmonary remodeling events. Eosinophil recruitment, activation and degranulation are prominent components of asthma exacerbations. Although eosinophil degranulation has historically been viewed as eliciting tissue damage, current research suggests that eosinophils also play roles in immunomodulation, tissue remodeling and host defense. We show here that eosinophils and their degranulation products, in a model of severe asthma, protect mice from the lethal sequelae of acute respiratory virus infection.

**Methods:** Eotaxin-2/interleukin-5 double transgenic (B6-E2IL5tg) mice (Ochkur, et al. J Immunol., 2007) were inoculated with the mouse pathogen, pneumonia virus of mice (PVM) at a dosage that is lethal for wild-type mice. Controls included B6 wild-type, B6-E2 and B6-IL-5 single transgenic mice and eosinophil-deficient B6- $\Delta$ dblGATA and B6- $\Delta$ dblGATA-E2IL5tg mice. Mice were evaluated in a survival study or sacrificed at 4 days post-inoculation for recovery of bronchial alveolar lavage fluid (BALF) and lung tissue. The cellular composition of BALF was determined morphologically using DiffQuik staining of cyto-spin preparations. Degranulation was measured by evidence of either eosinophil peroxidase using a specific ELISA or RNase activity and detection of immunoreactive mEARs in BALF. RNA was isolated from lung tissue and virus recovery was measured using a quantitative PCR assay directed against the virus SHPVM protein. Results from the B6-E2IL5tg asthma model were compared to those obtained from *Aspergillus fumigatus* sensitization and challenge of B6 wild-type mice.

**Results:** The B6-E2IL5tg mice display airways eosinophilia (90% of the cells recovered) including significant levels of eosinophil degranulation (i.e., activation). These mice also display histopathological pulmonary changes, airway dysfunction, and lung remodeling events associated with severe asthmatic patients. Infection of B6-E2IL5tg mice with the PVM pathogen resulted in a 200-fold decrease in virus recovery compared to B6-wild-type control mice; virus recovery remained unchanged in single transgenic (B6-E2tg or B6-IL-5tg) mice as well as in the eosinophil-deficient B6- $\Delta$ dblGATA, and cytokine-enriched but eosinophil-deficient B6- $\Delta$ dblGATA-E2IL5tg mice. Introduction of eosinophils into the airways of B6- $\Delta$ dblGATA-E2IL5tg mice by intratracheal instillation followed by PVM inoculation also resulted in a diminished virus recovery. Moreover, B6-E2IL5tg mice survive a PVM infection which is lethal to B6 wild-type, B6-E2tg, and eosinophil-deficient B6- $\Delta$ dblGATA, and B6- $\Delta$ dblGATA-E2IL5tg mouse strains. Interestingly, although the *A. fumigatus* sensitization and challenge model of allergic airways disease elicited eosinophil recruitment, there was no evidence of significant degranulation or cytokine release prior to virus challenge. Challenge with the PVM pathogen elicited release of ribonucleases in association with antiviral activity.

**Conclusions:** Activated eosinophils in the lung microenvironment protect mice from the lethal sequelae of infection with a respiratory virus pathogen.

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**ADOPTIVE TRANSFER OF ACTIVATED PULMONARY EOSINOPHILS IS SUFFICIENT TO RESTORE TH2 PULMONARY INFLAMMATION IN EOSINOPHIL-DEFICIENT PHIL MICE IN A MODEL OF ACUTE ASTHMA**

Elizabeth A. Jacobsen<sup>1</sup>, Dana Colbert<sup>2</sup>, Katie R. Zellner<sup>1</sup>, Nancy A. Lee<sup>2</sup>, and James J. Lee<sup>1</sup>

<sup>1</sup>Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

<sup>2</sup>Divisions of Haematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

**Rationale:** The Th2 character of allergic asthma is attributed to the activities of eosinophils, dendritic cells, macrophage, T cells and mast cells in the pulmonary and lymphatic compartments. The distinct functions of these cells have been difficult to delineate due to the complex interactions between these cells and the local stromal environment of the lung. Consequently, the roles of eosinophils in modulating the adaptive and innate immune responses in asthma remain poorly defined. The use of eosinophil-deficient mice and adoptive transfer techniques has suggested that eosinophils modulate the Th2 microenvironment through several mechanisms. In particular, our data suggests that pulmonary eosinophils, once activated, contribute to the recruitment and activation of both Th2 adaptive and innate immune cells in the lung in mouse models of asthma.

**Methods:** Eosinophil-deficient PHIL or (IL-5<sup>-/-</sup>) knockout mice received adoptive transfer of purified blood-derived eosinophils that were either untreated or cytokine treated (cultured 24-48 hrs with GM-CSF, IL-4, and IL-33) prior to transfer. Eosinophils were adoptively transferred directly into the lung (i.e., intratracheal instillation (i.t.)) into mice each day of allergen challenge using an established acute ovalbumin (OVA) sensitization/challenge protocol. Allergen-induced pulmonary changes were assessed as changes in Th2 cytokine levels, histopathologies, and monocyte and T cell populations by flow cytometry.

**Results:** Neither IL-5<sup>-/-</sup> or PHIL mice develop pulmonary pathologies nor leukocyte recruitment upon allergen sensitization/challenge alone, as described in previous studies. Adoptive transfer (i.t) of untreated blood-derived eosinophils into OVA sensitization/challenge mice restored pulmonary pathologies and T cell recruitment in IL-5<sup>-/-</sup> mice and not PHIL mice. Significantly, adoptive transfer (i.t) of cytokine treated eosinophils into OVA-sensitized/challenged PHIL mice was able to restore allergen-induced Th2 pulmonary pathologies and lung-specific accumulation of activated dendritic cells and T cells.

**Conclusion:** Our data demonstrates that eosinophils are a necessary component of the Th2 inflammatory response in an acute mouse model of asthma. In particular, we demonstrate that the immune microenvironment (GM-CSF, IL-4, IL-33) likely contributes to the activation of eosinophils, which then amplify the Th2 immune response to allergen. Collectively, these studies highlight an underappreciated role for eosinophils in amplification of the Th2 pulmonary immune responses that occur during allergen provocation.

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**IMPAIRED P2X1 RECEPTOR FUNCTION IN EOSINOPHILS FROM ASTHMATIC PATIENTS**

Catherine Vial<sup>1</sup>, Fiona Symon<sup>2</sup>, Martyn Mahaut-Smith<sup>1</sup>, Adam Wright<sup>2</sup>, Nicolas Sylvius<sup>3</sup> Michelle Muessel<sup>2</sup>, Peter Bradding<sup>2</sup> and Andrew Wardlaw<sup>2</sup>

Departments of <sup>1</sup>Cell Physiology and Pharmacology, <sup>2</sup>Infection, Immunity and Inflammation (Institute for Lung Health) and <sup>3</sup>Genetics, University of Leicester, UK.

**Background:** Extracellular nucleotides modulate eosinophil functions via metabotropic P2Y receptor activation. The present study investigates if P2X receptors, another type of receptor for extracellular nucleotides, are also involved in eosinophil regulation.

**Methods:** Human eosinophils were isolated from the blood of normal and asthmatic volunteers. Quantitative PCR (qPCR) was used to determine the level of P2X receptor mRNA expression in eosinophils from healthy and asthmatic donors, while P2X1 receptor protein expression level was investigated by Western blot. Conventional whole cell patch-clamp experiments were performed to identify the functional subtypes of P2X receptors in eosinophils. Platelet P2X1 receptor activity was assessed by measuring changes in intracellular calcium concentration  $[Ca^{2+}]_i$  after receptor stimulation. The eosinophil ATP content was quantified using a luciferin-luciferase assay.

**Results:** qPCR showed that P2X1, P2X4 and P2X5 receptors transcripts were expressed in eosinophils from three healthy and three asthmatic donors. Expression of P2X2, P2X3, P2X6 and P2X7 receptors were not confidently detected. The application of ATP (100mM) elicited a rapidly activating and rapidly desensitizing inward current ( $109.1 \pm 14.2$  pA/pF; donors=3, n=15 cells) in healthy human eosinophils which was abolished by 1mM of the selective P2X1 receptor antagonist NF449 ( $3.8 \pm 0.7$  pA/pF; donors=3, n=11 cells,  $p < 0.0001$ ). The P2X1 receptor agonist a,b-meATP (10mM) also induced a fast transient current in eosinophils ( $14.9 \pm 2.0$  pA/pF; donors=5, n=24 cells) which was totally inhibited by 1mM NF449 ( $1.2 \pm 0.9$  pA/pF; donors=4, n=17 cells,  $p < 0.0002$ ). These P2X1-like currents were markedly reduced (~75 %) in eosinophils from asthmatic donors compared to healthy donors (10mM a,b-meATP-induced currents of  $5.7 \pm 0.8$  pA/pF [donors=6, n=44 cells] and  $20.5 \pm 2.3$  pA/pF [donors=7, n=41 cells], for asthmatic and healthy eosinophils respectively,  $p < 0.0001$ ). Expression of the P2X1 receptor was the same in asthmatic and normal eosinophils by qPCR (mRNA) and western blotting (protein). Interestingly, the eosinophil ATP content was higher in asthmatic than in healthy donors ( $14.0 \pm 2.5$  and  $8.2 \pm 1.2$  ng of ATP/ $10^4$  cells respectively for asthmatic and healthy [13-14 donors],  $P < 0.05$ ). P2X1 receptor impaired function in asthmatic seems to be limited to eosinophils as a,b-meATP (10mM) induced a similar fast and transient  $[Ca^{2+}]_i$  rise in platelets from both healthy and asthmatic donors (5 donors for each).

**Conclusions:** This study shows that healthy human eosinophils express functional P2X1 receptors while asthmatic eosinophils express defective P2X1 receptors. The defective function of P2X1 is limited to eosinophils and could partly result from an increased release of ATP from asthmatic eosinophils. Impaired function of P2X1 could represent a biomarker of eosinophil activation in asthma.



**DYSREGULATED SYNTHESIS OF PROTECTIN D1 IN EOSINOPHILS FROM PATIENTS WITH SEVERE ASTHMA**

Jun Miyata<sup>1,2</sup>, Koichi Fukunaga<sup>1</sup>, Koichiro Asano<sup>1</sup>, Makoto Arita<sup>2</sup>

<sup>1</sup>Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Tokyo, <sup>2</sup>Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo

**Background:** Eosinophils are capable of synthesizing pro-inflammatory lipid mediators, such as leukotriene (LT) B<sub>4</sub>, LTC<sub>4</sub>, platelet activating factor (PAF), prostaglandin (PG) E<sub>2</sub>, and PGD<sub>2</sub>, which act as a positive feedback machinery in eosinophilic inflammation. It is, however, unclear whether this cell type possesses an inhibitory, self-regulatory system, and if it exists, whether it is dysfunctional in diseases associated with a persistent eosinophilic inflammation, such as severe asthma. Protectin D1 (PD1) is an anti-inflammatory, pro-resolving lipid mediator biosynthesized from the omega-3 fatty acid, docosahexaenoic acid (DHA) via 15-lipoxygenase. Exogenous PD1 conferred protection against eosinophilic inflammation in experimental asthma, though its endogenous cellular source and functions in human airways are of interest.

**Objective:** To investigate the synthesizing capacity of PD1 in eosinophils from healthy subjects and patients with severe asthma and its direct effects on eosinophil functions.

**Methods:** We used a CD16-negative and CD16-positive selection method to isolate eosinophils from the peripheral blood of healthy volunteers and patients presenting with severe asthma. Severe asthma was defined as according to the American Thoracic Society workshop consensus for definition of severe/refractory asthma. Human eosinophil-derived metabolites of arachidonic acid and DHA were analyzed with liquid chromatography-tandem mass spectrometry-based lipidomic analysis. The biological activities of PD1 on the function of human eosinophils, including chemotaxis, adhesion molecule expressions, degranulation, superoxide anion generation, or survival, were examined.

**Results:** The most abundant mediators of cyclooxygenase and 5-lipoxygenase synthesized in human eosinophils were thromboxane B<sub>2</sub> and LTC<sub>4</sub>, respectively. 15-hydroxy-eicosatetraenoic acid (HETE) was the main among the metabolites of 15-lipoxygenase, while modest amounts of the metabolite 17-hydroxy DHA was detected. We identified PD1 as one of the main anti-inflammatory, pro-resolving molecules synthesized in human eosinophils in presence of DHA. We found little synthesis of PD1 in the polymorphonuclear cell fraction of the peripheral blood, consisting mostly of neutrophils. PD1, in nanomolar concentrations, suppressed the chemotaxis induced by CCL11/eotaxin-1 or 5-oxo-eicosatetraenoic acid, and modulated the expression of the adhesion molecules, CD11b and L-selectin, though had no effects on the degranulation, superoxide anion generation, or survival of the eosinophils. Compared with the cells harvested from healthy subjects, we observed a prominent decrease in the biosynthesis of PD1 by eosinophils from patients with severe asthma, even in presence of DHA. There was also a significant decrease, in their eosinophils, in the levels of 15-HETE, a molecule synthesized via 15-lipoxygenase, using arachidonic acid as the substrate.

**Conclusion:** These observations are a first indication that activated human eosinophils represent a major source of PD1, which can act as a self-resolving machinery in eosinophilic inflammation, whereas production of PD1 and 15-lipoxygenase activity in human eosinophils are impaired in severe asthma.

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## ORTHOTOPIC HEART TRANSPLANTATION IN PATIENTS WITH EOSINOPHIL GRANULOMATOSIS WITH POLYANGIITIS : REPORT ON NINE CASES

Matthieu Groh<sup>1</sup>, Gabriella Masciocco<sup>2</sup>, Elizabeth Kirchner<sup>3</sup>, Arnt Kristen<sup>4</sup>, Carlo Pellegrini<sup>5</sup>, Shaïda Varnous<sup>6</sup>, Guillermo Bortman<sup>7</sup>, Mark Rosenberg<sup>8</sup>, Antonio Brucato<sup>9</sup>, Paul Waterworth<sup>10</sup>, Edgardo Bonacina<sup>11</sup>, Fabio Facchetti<sup>12</sup>, Leonard Calabrese<sup>3</sup>, Gina Gregorini<sup>13</sup>, Juan Jose Scali<sup>14</sup>, Randall Starling<sup>15</sup>, Maria Frigerio<sup>2</sup>, Andrea d'Armini<sup>5</sup>, Loïc Guillemin<sup>1</sup> for the French Vasculitis Study Group.

<sup>1</sup> Department of Internal Medicine, National Referral Center for Rare Autoimmune and Systemic Diseases (including Vasculitis, Scleroderma), INSERM U1016, Hôpital Cochin, APHP, Université Paris Descartes, Paris, France.

<sup>2</sup> Department of Cardiology and Heart Transplantation, Ospedale Niguarda, Milan, Italy.

<sup>3</sup> Department of Rheumatologic and Immunologic Disease, Cleveland Clinic, Cleveland, Ohio, USA.

<sup>4</sup> Department of Cardiology, Angiology, and Respiratory Medicine (AK), University Hospital Heidelberg, Heidelberg, Germany.

<sup>5</sup> Department of Cardiothoracic Surgery, Foundation IRCCS, Policlinico San Matteo, Pavia University school of medicine, Pavia, Italy.

<sup>6</sup> Department of cardiovascular and thoracic surgery, Hôpital de la Pitié Salpêtrière, APHP, Université Pierre et Marie Curie, Paris, France.

<sup>7</sup> Department of Cardiovascular Surgery, Sanatorio de La Trinidad Mitre, Buenos Aires, Argentina.

<sup>8</sup> Department of Internal Medicine III (Cardiology and Angiology), University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany.

<sup>9</sup> Department of Internal Medicine, Ospedale Papa Giovanni XXIII, Bergamo, Italy.

<sup>10</sup> Department of Cardiothoracic Surgery, Wythenshawe Hospital, Manchester, United Kingdom.

<sup>11</sup> Department of Pathology, Ospedale Niguarda, Milan, Italy.

<sup>12</sup> Department of Pathology, University of Brescia, Spedali Civili Brescia, Brescia, Italy.

<sup>13</sup> Division of Nephrology, Spedali Civili Brescia, Brescia, Italy.

<sup>14</sup> Department of Rheumatology, Autoimmune and Metabolic Bone Diseases, Durand Hospital, Buenos Aires, Argentina.

<sup>15</sup> Heart Failure Center, Heart & Vascular Institute, Cleveland Clinic, Cleveland, Ohio, USA.

**Background:** Heart involvement is the leading cause of death of patients with eosinophilic granulomatosis with polyangiitis (EGPA, Churg–Strauss syndrome) and is more frequent in antineutrophil cytoplasm antibody (ANCA) negative patients. Since disease remission is not achieved for all patients and that relapses are frequent, orthotopic heart transplantation (OHT) feasibility in EGPA patients could be addressed. In such settings, transplant outcome has only been reported once.

**Methods:** We conducted an international retrospective study of patients who underwent OHT for EGPA-related cardiomyopathy between October 2000 and December 2009.

**Results:** Nine patients were identified. All fulfilled the American College of Rheumatology criteria for EGPA. All but 2 had histologic evidence of vasculitis or clinical surrogates. All were ANCA- and had acute congestive heart failure due to severe eosinophilic myocarditis (mean troponin I  $14.6 \pm 20.5 \mu\text{g/L}$  and left ventricular ejection fraction (LVEF)  $24 \pm 6\%$ ). EGPA and EGPA-related cardiomyopathy diagnoses were concomitant for 5 patients and were separated by 12–21 months for the remaining 4. Before the graft, all patients received corticosteroids (CS) but only 5 had other immunosuppressants and only 3 received cyclophosphamide (CYC) pulses. Despite ongoing treatment, 6 (67%) patients' explanted hearts showed histologic evidence of EGPA. After survival lasting 3–60 months, 4 patients (44%) died of sudden deaths post OHT. Follow-up for the 5 (56%) survivors is 55–102 months. Three (33%) patients experienced EGPA relapses within 2–48 months post OHT and 3 additional patients required increased steroid doses for asthma and/or sinusitis exacerbations. The 5-year survival rate of EGPA patients transplanted since 2002 is similar to that reported by the International Society of Heart and Lung Transplantation (ISHLT) registry for the the same period.

**Conclusion:** It is essential to search for EGPA cardiac involvement early. Prompt treatment with CS and CYC is mandatory and may achieve recovery of the cardiac function. In refractory patients, OHT can be performed in accordance with ISHLT guidelines. After OHT, recent findings suggest that tacrolimus and mycophenolate mofetil are respectively superior to micro emulsion cyclosporin A and azathioprine. However, information on tacrolimus and mycophenolate mofetil use to treat EGPA patients is scarce. No optimal immunosuppressive strategy has been identified. Immunosuppression tapering post OHT is often difficult, because of EGPA relapses. Patients require close monitoring for arrhythmia after the graft, since sudden deaths occurred frequently. More data are needed.

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**PREDICTORS OF RESPONSIVENESS TO IMATINIB IN HYPEREOSINOPHILIC SYNDROME**

Paneez Khoury<sup>1</sup>, Amara G. Pabon<sup>1</sup>, JeanAnne M. Ware<sup>1</sup>, Roger Kurlander<sup>2</sup>, Irina Maric<sup>2</sup>, Amy D. Klion<sup>1</sup>.

<sup>1</sup>National Institutes of Allergic and Infectious Diseases, National Institutes of Health

<sup>2</sup>Division of Laboratory Medicine, Clinical Center, National Institutes of Health

**Background:** Although the efficacy of imatinib in the treatment of *PDGFRA*-negative HES is reported to be as high as 20%, predictors of response have not been described.

**Methods:** Subjects with HES meeting at least one of three criteria: 1) presence of the FIP1L1/*PDGFRA* fusion gene, 2) disease refractory to corticosteroids or 3) 4 or more laboratory criteria suggestive of a myeloproliferative presentation, were prospectively recruited for study. Laboratory criteria suggestive of a myeloproliferative disorder included anemia, thrombocytopenia, dysplastic eosinophils, abnormal megakaryocytes, spindle shaped mast cells, elevated serum tryptase and elevated serum B12. All subjects were treated with 400mg of imatinib for at least one month, at which time clinical, molecular, hematological, and bone marrow responses to imatinib were assessed. The primary outcome measure was a decrease in eosinophil count by 50% at one month.

**Results:** All 12 subjects with the FIP1L1/*PDGFRA* fusion gene responded to imatinib therapy with normalization of eosinophil counts within 1 week and improvement of bone marrow abnormalities at one month following initiation of therapy. Although complete resolution of eosinophilia took a median of 5 weeks (range 1-12 weeks), 5/9 *PDGFRA*-negative subjects meeting myeloproliferative criteria demonstrated a 50% decrease in eosinophil count at 1 month. In contrast, none of the 6 corticosteroid-refractory subjects who lacked myeloproliferative features met the primary outcome measure ( $p < 0.05$  as compared to either of the other groups).

**Conclusions:** Our data suggest that the presence of clinical and laboratory features of myeloproliferative disease is a predictor of imatinib responsiveness in patients with *PDGFRA*-negative HES. However, unlike patients with *PDGFRA*-positive disease who respond rapidly to low doses of imatinib, *PDGFRA*-negative patients should be treated with 400 mg daily for a minimum of 4 weeks before drug failure is determined.

**Grant support:** *Division of Intramural Research, NIH*

## POSTER I

**RAPAMYCIN POTENTLY AND SPECIFICALLY INHIBITS IL-5+ TH2 CELL PROLIFERATION BY A MTORC1/S6 KINASE1 DEPENDENT MECHANISM**Yuzhi Yin<sup>1</sup> and Calman Prussin<sup>1</sup><sup>1</sup>Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

**Background:** We have previously shown that IL-5 expression is limited to a minority subpopulation of highly differentiated pro-eosinophilic IL-5+ Th2 cells. These IL-5+ Th2 cells are associated with eosinophilic GI disease (EGID) and their frequency is highly correlated with peripheral eosinophilia. In screening for potential anti-Th2 therapeutic targets, we examined rapamycin, an inhibitor of the mTOR (mechanistic target of rapamycin) kinase.

**Methods:** House dust mite (HDM) or respiratory syncytial virus (RSV) antigen-specific proliferation of Th2 and Th1 cells, respectively, were examined in peripheral blood mononuclear cells from allergic asthmatic donors using dye dilution, intracellular cytokine staining, and flow cytometry. Th2 responses were further characterized as IL-5+ Th2 (IL-5+, IL-13+) and IL-5- Th2 (IL-5-, IL-13+). *In vitro* differentiated Th1 and Th2 cell lines were generated from naïve CD4 T cells using published methods. Western blotting and intracellular staining were used to measure the phosphorylation of mTOR downstream targets, S6 ribosomal protein (rp), 4E-binding protein (BP), and AKT.

**Results:** CD4 T cells that proliferated to RSV and HDM were dominantly Th1 and Th2, respectively. Over all concentrations examined (0.25, 0.5, 1.0, 10 nM), rapamycin inhibition of HDM specific Th2 proliferation was greater than that of RSV specific Th1 responses ( $p < 0.001$ ). Additionally, the inhibition of IL-5+ Th2 responses was significantly greater than IL-5- Th2 ( $p = 0.009$ ). At 1 nM rapamycin, IL-5+ Th2, IL-5- Th2, and Th1 responses were inhibited 97%, 90%, and 51%, respectively ( $p = 0.009$ ). Similarly, rapamycin inhibition of anti-CD3-induced proliferation of Th2 cell lines was greater than that of Th1 cell lines (92 vs. 59 %, respectively). Rapamycin inhibited the anti-CD3 induced phosphorylation of S6rp in Th2 cell lines to a greater extent than in Th1 cell lines (74% vs. 46%, respectively). In Th2 cells, phosphorylation of 4E-BP or AKT was not induced by anti-CD3 nor affected by rapamycin.

**Conclusions:** Rapamycin potently and preferentially inhibits Th2 cell proliferation relative to Th1 cells, as demonstrated in both *ex vivo* as well as *in vitro* differentiated effector T cells. Notably, this Th2 inhibition was greatest for the “pro-eosinophilic” IL-5+ Th2 subpopulation. This preferential inhibition of Th2 proliferation was paralleled by greater inhibition of S6rp phosphorylation, suggesting that rapamycin inhibits Th2 cell proliferation through inhibition of S6 kinase1. At the concentrations used, rapamycin inhibited phosphorylation of S6rp, but not AKT, demonstrating that this Th2 specific effect is operating by inhibiting mTOR complex 1 (mTORC1) activity. These findings further establish IL-5+ Th2 cells as a unique Th2 subpopulation with specific metabolic and signaling requirements.

Rapamycin potently inhibited Th2 responses at sub-nanomolar concentrations. This suggests that rapamycin may have anti-Th2 therapeutic effects at substantially lower doses than the 5-15 nM that is typically used in transplantation. Furthermore, the greater sensitivity of IL-5+ Th2 cells to rapamycin inhibition suggests that rapamycin and the mTORC1 pathway may be a potential therapeutic targets for Th2 driven eosinophilic disease.

**Grant support:** NIAID Division of Intramural Research, project AI000993-06.

## POSTER 2

### PUTTING BRAKES ON EOSINOPHIL DEVELOPMENT: RHOH IN HEALTH AND DISEASE

Christina Stoeckle, Barbara Geering, Nicola Andina, Hans-Uwe Simon

Institute of Pharmacology, University of Bern, 3010 Bern, Switzerland

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

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

**Methods:** Eosinophils were isolated from peripheral blood of healthy donors or HES patients and RhoH expression analysed by western blot. Haematopoietic stem cells and immature granulocytes were isolated from human bone marrow, differentiated into eosinophils and analysed by western blot. Bone marrow was isolated from RhoH<sup>-/-</sup> or wild type mice and cultured with Flt-3L, SCF and IL-5 to induce differentiation into eosinophils (bmEos; according to Dyer K et al. JI 2008). Blood, bone marrow and bmEos were stained with Diff Quick and analysed by light microscopy or stained with fluorescently labelled antibodies and analysed by flow cytometry.

**Results:** We find that RhoH expression is dysregulated in HES patients. In contrast to what has been reported for T cells, we find that lack of RhoH promotes eosinophil development and differentiation, resulting in increased eosinophil numbers and faster maturation which is accompanied by an altered eosinophil phenotype.

**Conclusions:** The results suggest a regulatory role of RhoH in eosinophil development and function, possibly through regulation of growth factor signalling and/or alterations in transcription factors that regulate granulopoiesis.

**Grant support:** *This work is funded by the Deutsche Forschungsgemeinschaft (German Research Foundation; DFG) and the Swiss National Science Foundation (SNF).*

## POSTER 3

**THYMIC STROMAL LYMPHOPOIETIN PROMOTES HUMAN EOSINOPHIL-BASOPHIL LINEAGE COMMITMENT: A KEY ROLE FOR TUMOR NECROSIS FACTOR-ALPHA**

Claudia C.K. Hui<sup>1</sup>, Sina Rusta-Sallehy<sup>1</sup>, Delia Heroux<sup>1</sup>, and Judah A. Denburg<sup>1</sup>

<sup>1</sup>Division of Allergy & Clinical Immunology, Department of Medicine, McMaster University

**Rationale:** Allergic diseases are characterized by tissue eosinophilic and basophilic inflammation. Both epithelial-derived thymic stromal lymphopoietin (TSLP) and eosinophil/basophil (Eo/B) lineage-committed progenitor cells are upregulated and found at sites of allergic inflammation. We have previously shown that TSLP mediates the differentiation of peripheral blood (PB) CD34+ progenitor cells into eosinophils and basophils. However, the specific mechanisms through which TSLP promotes this lineage commitment are unclear.

**Objectives:** We aim to characterize the intracellular mechanisms by which TSLP mediates Eo/B differentiation.

**Methods:** Purified CD34+ progenitor cells were isolated from fresh PB using a progenitor cell enrichment kit via negative selection magnetic-activated cell sorting techniques. PB CD34+ cells were stimulated overnight with media, IL-3 (1 ng/mL), TSLP (10 ng/mL), or IL-3/TSLP and assessed for cytokine and chemokine secretion using Luminex assays. Alterations in Eo/B colony forming units (CFU) and surface expression of TSLPR post-stimulation with IL-3/TSLP (and/or neutralizing anti-TNF- $\alpha$  Ab) were assessed by methylcellulose cultures and flow cytometry respectively.

**Results:** TSLP alone induced significant levels of IL-1 $\beta$  (p<0.05), IL-6 (p<0.001) and CXCL8 (p<0.001) from PB CD34+ cells, compared to unstimulated controls. IL-3/TSLP-stimulated CD34+ cells released significant levels of IL-1 $\beta$ , IL-6, IL-10, IL-13, TNF- $\alpha$ , CXCL8 and CCL2, but failed to secrete detectable levels of IL-4, IL-9, GM-CSF, IFN- $\gamma$ , and eotaxin. Blockade of TNF- $\alpha$  *in vitro* in the differentiation assays inhibited both TSLPR expression (p<0.05) and IL-3-responsive Eo/B CFU formation (p<0.01). Overnight stimulation of PB CD34+ cells with IL-3 (10 ng/mL) and TNF- $\alpha$  (50 pg/mL) enhanced surface expression of TSLPR to comparable levels post TSLP/IL-3-stimulation. Moreover, pre-stimulating CD34+ cells with IL-3/TNF- $\alpha$  overnight prior to culturing in methylcellulose cultures resulted in enhanced sensitivity to TSLP-mediated Eo/B colony formation at lower concentrations of TSLP (1 ng/mL), which was statistically significant compared to IL-3-stimulated CD34+ cells (p<0.001).

**Conclusion:** We have previously shown that stimulation of human PB CD34+ cells with TSLP promotes Eo/B differentiation through upregulation of IL-3R $\alpha$  and TSLPR. Our current study demonstrates that TSLP can modulate Eo/B lineage commitment, by inducing PB CD34+ cells to actively secrete chemokines and cytokines (key among which is TNF- $\alpha$ ), which, together with IL-3, induce the upregulation of TSLPR, leading to the subsequent amplification of Eo/B CFU. The novel role of TSLP-induced Eo/B differentiation points to the importance of the epithelium, and its responses to environmental stimuli, in the development of allergic diseases.

**Grant support:** *This work is funded by the CIHR, AllerGen NCE (CAIDATI Award), McMaster University (Medicine Graduate Initiative Fund)*

**POSTER 4****IN VIVO EOSINOPHIL LABELING SHOWS A CIRCULATORY LIFESPAN OF 66-84 HOURS**

Tamar Takl, Kiki Tesselaar<sup>2</sup>, José A. Borghans<sup>2</sup>, Leo Koenderman<sup>1</sup>

<sup>1</sup>Department of Respiratory Medicine, University Medical Centre Utrecht, Utrecht, the Netherlands

<sup>2</sup>Department of Immunology, University Medical Centre Utrecht, Utrecht, the Netherlands

**Background:** A remarkable lack of knowledge is present regarding the kinetics of inflammatory effector cells during normal immune homeostasis and under (allergic) diseased conditions, both in blood and tissue. This is nicely illustrated by a seemingly long retention of these cells in lung tissue during anti-IL-5 treatment, which is characterized by eosinopenia in both blood and sputum compartments. It is difficult to reconcile these studies with a short lifespan of eosinophils.

**Objectives:** To determine the lifespan of granulocytes in peripheral blood and tissues in humans using *in vivo* deuterium (<sup>2</sup>H) labeling.

**Methods:** We applied a technique that allowed the study of untouched granulocytes in humans without *ex vivo* manipulation. Healthy volunteers took oral doses of <sup>2</sup>H-glucose, which was incorporated in the DNA of all dividing cells. Hereafter, small blood samples were taken and different cell populations were obtained by high performance cell sorting. DNA of these cells was isolated and its relative content of <sup>2</sup>H/<sup>1</sup>H determined by a combination of gas-chromatography and mass spectrometry. In combination with computational modelling that takes into account both blood and bone marrow compartments, this technique allowed the determination of the circulatory lifespan of both neutrophils and eosinophils.

**Results:** The first labeled neutrophils appeared in the peripheral blood after 6 days, eosinophils appear after 3 days, which indicated differences in post mitotic pool (PMP) transit times between both cell types. Simulation of the kinetics of <sup>2</sup>H-glucose incorporation in peripheral blood leukocytes showed greatest similarities to models with a neutrophil blood lifespan of 52-68 hours and an eosinophil lifespan of 66-84 hours.

**Discussion:** The blood life spans of neutrophils and eosinophils differ, but are both in the order of days rather than hours. The pulse-chase design might, however, have been biased by a relatively high labeling of a fast cycling pool of progenitors, which might have lead to an underestimation of the lifespan of the total population. On the other hand, retention of label in the bone marrow might have lead to an overestimation of the circulatory lifespan.

**Grant support:** *This work is funded by the Dutch Lung Foundation, grant #10.052*

## POSTER 5

**DIFFERENTIAL PROMOTER USAGE AND REGULATION OF THE HUMAN IL-5RA GENE IN DEVELOPING EOSINOPHIL PROGENITORS**

Fan K. Gao, Jian Du, Steven J. Ackerman

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

**Background:** Interleukin-5 receptor  $\alpha$  (IL-5R $\alpha$ ) is a critical component of the IL-5 signaling pathway responsible for the proliferation and differentiation of eosinophil progenitors required for the development of eosinophilia, and the recruitment, activation and survival of the eosinophil in tissues. Our group and others previously identified two promoters, P1 and P2, which are functionally active in eosinophil-differentiated cell lines. However, there is little understanding of the roles of these two promoters in terms of their differential usage and regulation during the development of authentic eosinophil progenitors. Prior studies have implicated combinatorial interactions of transcription factors including GATA-1, PU.1, C/EBP family members and their associated proteins in transcriptional control of eosinophil specific genes. In the current study, we demonstrate that the human IL-5R $\alpha$  P1 and P2 promoters are under differential temporal regulation during the development of authentic umbilical cord blood-derived CD34<sup>+</sup> eosinophil progenitors as mediated by differential occupancy by relevant transcription factors known to be essential in this process.

**Methods:** Human umbilical cord blood-derived CD34<sup>+</sup> hematopoietic progenitors under IL-5-induced eosinophil differentiation were transiently transfected with IL-5R $\alpha$ P1 or P2 promoter luciferase reporter constructs at various time points to assay differential promoter activity during eosinophilopoiesis. *In silico* prediction of transcription factor binding sites was matched to results from DNase I *in vivo* footprinting with ligation-mediated PCR (LM-PCR) to identify potential transcription factors regulating the two promoters. Subsequently, chromatin immunoprecipitation (ChIP) assays were performed on the differentiating CD34<sup>+</sup> progenitors to determine the dynamic occupancy of the identified transcription factors on the two promoters during eosinophil differentiation.

**Results:** The IL-5R $\alpha$  P1 promoter is active throughout IL-5-induced eosinophil differentiation of CD34<sup>+</sup>/IL-5R $\alpha$ <sup>+</sup> eosinophil progenitors (EoPs). In contrast, the IL-5R $\alpha$  P2 promoter exhibits a sudden induction of activity early in differentiation (Day 7) to overtake P1 activity ( $p=0.04$ ) before being attenuated during continued eosinophil development. This sudden induction of P2 activity also coincides with a dramatic induction in levels of the predominant soluble IL-5R $\alpha$  splice variant mRNA. *In vivo* DNase I footprinting with LM-PCR revealed hypersensitive sites within P1 and P2 promoter regions that correspond to consensus binding sites for GATA-1, PU.1 and the C/EBP family of transcription factors (C/EBPs  $\alpha$ ,  $\beta$  and  $\epsilon$ ). Furthermore, quantitative ChIP analyses demonstrate that the P1 and P2 promoters exhibit differential occupancy by these transcription factors during the course of eosinophil development.

**Conclusions:** Whereas the human IL-5R $\alpha$  P1 promoter remains active throughout eosinophil differentiation, the P2 promoter exhibits a transient and higher activity than P1 early in differentiation. This potentially represents a "boost" needed by the early eosinophil progenitor to produce the coincidental high-level expression of the IL-5R $\alpha$  soluble isoform mRNA. Such temporal and independent regulation of the two promoters occurs through dynamic and combinatorial interactions of stage-specific transcription factors, and may provide a mechanism by which expression of the IL-5R $\alpha$  IL-5-binding soluble isoform is induced during eosinophil development to down-regulate IL-5 signaling to control continued expansion of the eosinophil lineage in parasitic and allergic diseases.

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**POSTER 6****REVEILING THE DEATH PATHWAY LEADING TO EOSINPHIL CYTOLYSIS**

Susanne Irene Radonjic-Hoesli

**Background:** Eosinophil cytolysis is considered as a mode of degranulation, leading to the release of intact granules, so-called clusters of free eosinophil granules (cfegs). It has been referred to as “state of utmost activation”, however, leads to eosinophil death. The appearance of cfegs has been demonstrated in eosinophilic tissues in different diseases. The mechanisms underlying this activation-induced form of cell death in eosinophils remain unclear.

**Methods:** Isolated blood eosinophils were incubated on glass cover slips in adhesion enhancing conditions to induce cytolysis. Morphological characterization of the distinct stages of the proposed cascade was addressed by means of electron microscopy, histology, and immuno-histochemistry. Experiments with pharmacological inhibitors were performed to underline the sequel of events within the cascade. Identification of molecular events involved in the initiation and progression were analyzed by examination of phosphorylation events associated with different signalling pathways using immuno-fluorescence.

**Results:** We propose a cascade of events, involving adhesion, granule fusion processes, reactive oxygen species (ROS) production, and earlier degranulation steps, leading to a distinct morphology characterized by cytosolic vacuolization. This cascade of events involves amplification steps and leads to cell death, which is characterized by loss of granule, vacuole, plasma, and nuclear membrane integrity. On a molecular level, we demonstrate a signalling cascade, involving the  $\beta$ 2-integrin Mac1, phosphatidylinositol 3-kinases (PI3K), p38 mitogen-activated kinase (MAPK), receptor-interacting serine/threonine-protein kinase 1 (RIPK1). Major effectors in the progression of cytosolic vacuolization to cytolysis are furthermore ROS, which are mitochondrial as well as NADPH oxidase derived.

**Conclusion:** Taken together, we report an adhesion-triggered programmed cell death pathway in eosinophils associated with the release of cfegs that represents at least one signalling cascade leading to the well known morphological phenomenon of eosinophil cytolysis.

## POSTER 7

**MECHANISM OF SIGLEC-8-MEDIATED CELL DEATH IN IL-5-ACTIVATED EOSINOPHILS: ROLE FOR ROS-ENHANCED MEK/ERK ACTIVATION**

Gen Kano<sup>1</sup>, Maha Almanan<sup>1</sup>, Bruce S. Bochner<sup>2</sup> and Nives Zimmermann<sup>1</sup>

<sup>1</sup>Division of Allergy & Immunology, Cincinnati Children's Hospital, and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA.

<sup>2</sup>Department of Medicine, Division of Allergy and Clinical Immunology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

**Background:** Siglec-8 is expressed on human eosinophils, where its ligation induces cell death. Paradoxically, Siglec-8-mediated cell death is markedly enhanced by the presence of the activation and survival factor IL-5 and becomes independent of caspase activity.

**Objective:** In this report we investigate the mechanism of Siglec-8-mediated cell death in activated eosinophils.

**Methods:** Human peripheral blood eosinophils were treated with anti-Siglec-8 antibody and IL-5, and mode of cell death was determined by flow cytometry and morphology. Phosphorylation of MAPK was determined by phospho-luminex, flow cytometry, and Western blotting. ROS (reactive oxygen species) accumulation was determined by dihydrorhodamine (DHR) fluorescence and EPX release was also monitored.

**Results:** Co-stimulation with anti-Siglec-8 and IL-5 significantly increased the rate and proportion of cells dying by necrosis accompanied by EPX release as compared to stimulation with anti-Siglec-8 alone, in which apoptosis predominated. Together with the caspase-independent mode of cell death in co-stimulated cells, these findings suggest the activation of a specific and distinct biochemical pathway of cell death during anti-Siglec-8/IL-5 co-stimulation. Phosphorylation of ERK1/2 and MEK1 was significantly enhanced and sustained in co-stimulated cells compared to cells stimulated with IL-5 alone; anti-Siglec-8 alone did not cause ERK1/2 phosphorylation. MEK1 inhibitors blocked anti-Siglec-8/IL-5-induced cell death. ROS accumulation was induced by Siglec-8 ligation in a MEK-independent manner. In contrast, preincubation with a ROS inhibitor prevented the anti-Siglec-8/IL-5-induced enhancement of ERK phosphorylation and cell death. Addition of exogenous ROS mimicked stimulation by anti-Siglec-8 and was sufficient to induce enhanced cell death in IL-5-treated cells. Collectively, these data suggest that the enhancement of ERK phosphorylation is downstream of ROS generation.

**Conclusions:** In IL-5-activated eosinophils, ligation of Siglec-8 leads to ROS-dependent enhancement of IL-5-induced ERK phosphorylation, which results in a novel mode of biochemically-regulated eosinophil cell death.

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## POSTER 8

**“SELF-RECOGNITION” VIA PAIRED IMMUNOGLOBULIN-LIKE RECEPTORS IS FUNDAMENTAL FOR EOSINOPHIL HOMEOSTASIS**

Ben Baruch-Morgenstern Neta<sup>1\*</sup>, Shik Dana<sup>1\*</sup>, Moshkovits Itay<sup>2\*</sup>, Itan Michal<sup>3\*</sup>, Bouffi Carine<sup>4</sup>, Fulkerson Patricia<sup>4</sup>, Rashkovan Diana<sup>5</sup>, Jung Steffen<sup>6</sup>, Rothenberg E. Marc<sup>7</sup>, Munitz Ariel<sup>8,1</sup>

\* -Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, The Tel-Aviv University, Ramat Aviv, 69978, Israel.

† - Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH, 45229

‡ - Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

^ - These authors contributed equally to this work

**Background:** A major checkpoint in curtailing cytotoxic T cells and natural killer cells is the inhibitory signaling mediated by major histocompatibility complex (MHC) restricted self-recognition. Whether this applies to other cells in the immune system has received little attention. We have recently shown that eosinophils express paired immunoglobulin-like receptors PIR-A and PIR-B, which bind MHC-I molecules and mediate activation and inhibition, respectively. In fact, PIR-B is a “prototype” immunoreceptor tyrosine-based inhibitory motif-containing receptor that is capable of suppressing activation signals elicited via PIR-A by recruitment of intracellular phosphatases such as SHP-1 and SHP-2. Yet, the precise roles of PIRs in eosinophils are unknown especially in context of “self recognition”.

**Materials and Methods:** Low-density bone marrow-derived (LDBM) eosinophils were generated from wild type (WT), *Pirb*<sup>-/-</sup> and *B2m*<sup>-/-</sup> mice. Eosinophil differentiation and viability were assessed during a 14-days culture (flow cytometry) and colony forming unit (CFU) assays were performed in response to IL-5, IL-3 and GM-CSF. Apoptosis of eosinophils in the BM of naïve, IL-5-treated and aeroallergen-challenged mice were determined (flow cytometry). WT and *Pirb*<sup>-/-</sup> mice were intranasally challenged with aeroallergen extracts. Bronchoalveolar lavage fluid was assessed for total and differential cell counts and Th2 cytokine/chemokine secretion (ELISA). Serum was assessed for total IgE (ELISA).

**Results:** LDBM cell cultures from *Pirb*<sup>-/-</sup> mice failed to produce mature eosinophils (but not other myeloid cells) in-vitro as determined by surface markers, cellular morphology and granule proteins. Following exposure to IL-5, the *Pirb*<sup>-/-</sup> LDBM cells underwent apoptosis as assessed by expression of Annexin-V and active caspase 3. CFU assays revealed that this phenomenon was specific to IL-5 but not IL-3 and/or GM-CSF. Consistently, naïve *Pirb*<sup>-/-</sup> mice displayed markedly elevated apoptotic eosinophils in the BM resulting in decreased baseline peripheral blood eosinophilia even in the presence of exogenous IL-5 delivery. Mechanistically, PIR-A neutralization suppressed the apoptotic phenotype of *Pirb*<sup>-/-</sup> cells and rescued the LDBM cultures to produce mature eosinophils. Moreover, *B2m*<sup>-/-</sup> mice (which lack both PIR-A and PIR-B signaling) had increased eosinophil CFU colony formation and decreased eosinophil apoptosis in-vitro and in-vivo. Aeroallergen-challenged *Pirb*<sup>-/-</sup> mice displayed markedly decreased lung eosinophilia, and an attenuated Th2 response. Decreased Th2-responses in aeroallergen-challenged *Pirb*<sup>-/-</sup> mice were likely driven by eosinophils since they were independent of alterations in allergic sensitization of PIR-B expression in CD11c<sup>+</sup> cells.

**Conclusion:** We demonstrate that PIR-B suppresses PIR-A-induced eosinophil apoptosis, which counterbalances survival and growth signals selectively driven by IL-5. Lack of MHC-I expression, which results in lack of PIR-A and PIR-B-induced signaling results in decreased homeostatic eosinophil apoptosis in the BM and increased eosinophil colony formation, likely due to loss of intrinsic PIR-A-induced apoptosis. Our data opens a new paradigm in the understanding of eosinophil homeostasis and may have significant implications for eosinophil-associated diseases.

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## POSTER 9

## OPTIMIZED PROTOCOLS FOR EOSINOPHIL PROGENITOR STUDIES

Kaila L. Schollaert<sup>1</sup>, Michael R. Stephens<sup>1</sup>, Marc E. Rothenberg<sup>1</sup> and Patricia C. Fulkerson<sup>1</sup>

<sup>1</sup>Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH

**Background:** Eosinophils are produced in the bone marrow from CD34+ eosinophil lineage-committed progenitors (EoP), whose levels in the bone marrow are elevated in a variety of diseases, suggesting that increased EoP production is an important process in disease-associated eosinophilia. EoP have been identified in murine and human bone marrow, but pathways central to the biology of the EoP remain unknown. Development of new methods to investigate regulators of EoP production and differentiation is needed to identify potential therapeutic targets that specifically inhibit eosinophil production.

**Methods:** EoP and eosinophils were identified using standard flow cytometry techniques. Lineage depletion of bone marrow was carried out by MACS or density centrifugation. Eosinophil cultures were performed in IMDM medium with 10% characterized FBS and antibiotics, and subjected to a cytokine regimen of different doses and combinations of FLT3L, SCF for the first 4 days, and IL-5 thereafter for an additional 10-11 days. WBM and LDBM cells were frozen in 90% FBS and 10% DMSO by immersion in a room-temperature isopropanol bath which was immediately stored at -80°C. Cultured mature eosinophil chemotactic responses were evaluated using 96-well 5-micron transwell plates.

**Results:** Murine BALB/c EoP are rare, comprising  $0.132 \pm 0.016\%$  of live whole bone marrow (WBM) (mean  $\pm$  SEM,  $n = 6$ ) or  $983 \pm 253$  EoP per million live WBM (mean  $\pm$  SEM,  $n = 3$ ). Purification of murine EoP by FACS yielded  $319 \pm 30$  cells per million sorted WBM (mean  $\pm$  SEM,  $n = 4$ ). Depletion of lineage-positive cells from WBM resulted in the greatest EoP enrichment (16-fold), but fractionation of WBM by centrifugation for low-density mononuclear cells (LDBM) was an acceptable alternative as EoP were enriched >2-fold. Stimulation of LDBM with SCF was necessary and sufficient for optimal eosinophil yield while addition of FLT3L provided no significant additive effect. Our optimized LDBM culture method yielded mature eosinophils that displayed a robust chemotactic response toward CCL11 and leukotriene B4. We also tested the efficiency of eosinophil yield from WBM that was frozen, thawed and then subjected to centrifugation for LDBM prior to culturing. There was no significant difference in eosinophil yield from LDBM derived from fresh or frozen WBM. In addition, mature eosinophils were also frozen and thawed to yield viable cells ( $94 \pm 3\%$  viability, mean  $\pm$  SD,  $n = 2$ ). We further determined the number of LDBM cells to seed in 96-well dishes for maximum eosinophil yield.

**Conclusions:** We have developed an optimized eosinophil culture protocol that enriches for EoP in LDBM starting population along with additional methods that represent new, powerful tools for murine EoP and eosinophil research and provide significant flexibility for experimental design.

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## POSTER 10

**EFFECTS OF HYPOXIA ON EOSINOPHIL DEGRANULATION, APOPTOSIS, AND SENSITIVITY TO GLUCOCORTICOSTEROIDS**

Linsey Porter<sup>1</sup>, Neda Fahari<sup>1</sup>, Andrew S. Cowburn<sup>1</sup>, Alison M. Condliffe<sup>1</sup>, Stuart Farrow<sup>2</sup> and Edwin R. Chilvers<sup>1</sup>

<sup>1</sup>Respiratory Medicine Division, Department of Medicine, University of Cambridge, Cambridge, UK;

<sup>2</sup>GlaxoSmithKline, Stevenage, UK

**Background:** Many tissues including the skin, intestinal epithelium and potentially the airway operate at 'physiological' levels of tissue hypoxia with PO<sub>2</sub> values consistently below 3KPa. Both sterile and non-sterile inflammation will exacerbate this physiological tissue hypoxia. These factors predicate the need for granulocytes including eosinophils to operate efficiently under hypoxia. The aim of this study was to determine whether hypoxia affects eosinophil survival and function given the pivotal role that eosinophils play in allergic various inflammatory processes.

**Methods:** Human peripheral blood eosinophils were prepared from healthy, atopic and asthmatic donors using hetastarch-sedimentation and EasySep®-immunomagnetic beads, and were cultured in RPMI + 10% autologous serum for 4-24 h. Apoptosis was quantified using standard morphology and AnnV/PI staining. Eosinophil secretory processes (IL-8 and eosinophil derived neurotoxin (EDN) release) were evaluated by ELISA. To determine whether the level of IL-8 release was physiologically relevant, eosinophil supernatants were assessed in a neutrophil chemotaxis assay. Neutrophils were purified from the peripheral blood of healthy volunteers using plasma-Percoll gradients.

**Results:** Hypoxic incubation (H) (media PO<sub>2</sub> 2.9 ± 0.07 KPa) prolonged eosinophil survival by delaying apoptosis when compared to normoxia (N) (% apoptosis at 24 h: N 16.28 ± 3.02 %; H 1.182 ± 0.21 %, n=5); this effect was of similar magnitude to that observed with IL-5 (H 0.42 ± 0.29 %). Most strikingly, hypoxic incubation also reduced the well documented pro-apoptotic effect of glucocorticoids (1 µM dexamethasone (Dex)) (% apoptosis at 24 h: N + Dex 27.56 ± 3.89%, H + Dex 11.18 ± 2.94% n=5). The hypoxic survival effect was recapitulated by hypoxia mimetics (the iron chelator DFO (10 mM) and the 2-oxyglutarate analogue DMOG (1 mM)), which also antagonised Dex-induced apoptosis, 11.57 ± 4.19 % and 17.93 ± 13.49 % respectively, (n=3). qPCR analysis of the glucocorticoid-dependent gene *GILZ* and the HIF-1α-dependent gene *GLUT1* demonstrated that the suppression of Dex-induced eosinophil apoptosis was not due to the inhibition of steroid-induced transcriptional activity (mRNA fold change at 16 h: *GILZ* N 12.89 ± 3.63, *GILZ* H 23.9 ± 9.24; *GLUT1* H (DMSO) 28.46 ± 7.18, *GLUT1* H (Dex) 45.5 ± 13.49, n=3).

Whilst hypoxia did not enhance the release of EDN over 4/ or 24 h, IL-8 release was increased significantly by hypoxic culture; this effect was most pronounced in cells from atopic subjects (pg/ml IL-8 release at 15 h in healthy controls: N 110 ± 76, H 295 ± 268, n=5; atopic subjects: N 450 ± 240, H 1765 ± 1001, n=9). Eosinophil supernatants were chemotactic to purified neutrophils and this effect was greater with supernatants generated under hypoxic conditions (number of migrated neutrophil per 0.1 µl in healthy controls: N 88 ± 56, H 145 ± 72; atopic subjects; N 108 ± 64, H 141 ± 72).

**Conclusions:** Hypoxia delays constitutive eosinophil apoptosis and attenuates dexamethasone-induced apoptosis. Furthermore, hypoxia enhances constitutive IL-8 release, potentially contributing to neutrophil recruitment. These findings suggest hypoxia will impede the *in vivo* resolution of eosinophil-driven inflammatory conditions such as asthma and may contribute to glucocorticoid-resistance in some settings.

## POSTER II

**DEXPRAMIPEXOLE DECREASES BLOOD EOSINOPHILS: RESULTS OF TWO CLINICAL TRIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS**

Michael E. Bozik<sup>1</sup>, Thomas Petzinger, Jr<sup>1</sup>, James L. Mather<sup>1</sup>, Gregory T. Hebrank<sup>1</sup>, Steven I. Dworetzky<sup>1</sup>, Ian J. Reynolds<sup>1</sup>, Mary Sullivan<sup>1</sup>, Wildon R. Farwell<sup>2</sup>

<sup>1</sup>Knopp Biosciences, Pittsburgh PA USA, <sup>2</sup>Biogen Idec, Cambridge, MA USA

**Background:** Prior clinical findings identified neutropenia as a potential adverse effect of dexamipexole (DEX) in patients with amyotrophic lateral sclerosis (ALS). In Phase II and Phase III studies of DEX as a potential treatment for ALS, white blood cell status was monitored as a component of routine clinical laboratory assessments and to assess neutropenia.

**Methods:** The Phase II study randomized 102 subjects and the phase III study randomized 943 subjects in double-blind, placebo controlled trials to assess the safety and efficacy of DEX in ALS, respectively. Subjects were randomized to 25mg, 75mg, or 150mg DEX twice daily or placebo for up to nine months (Phase II) or 150mg DEX twice daily or placebo for up to 18 months (Phase III). Monthly CBCs were obtained in both studies.

**Results:** In the two-part, Phase II study, a dose-dependent decrease in eosinophil count was observed following 12 weeks of treatment with DEX at doses of 25mg, 50mg, and 150mg twice daily versus placebo (part 1). Following a 4-week, single-blind drug washout, subjects in part 2 who were re-randomized to 150mg twice daily had a greater decline in eosinophils than subjects re-randomized to 25mg twice daily. The part 1 decrease in eosinophil count was partially reversed by the end of the 4-week washout period.

In Phase III, a profound decrease in blood eosinophil count was observed after 8-12 weeks of treatment with DEX that persisted for the duration of the trial. Counts were reduced by approximately 70% in the treated group, while there was a trend towards increased eosinophil count in patients receiving placebo. The effect was observed in most patients, with 82% of DEX treated subjects experiencing a 50% or greater decline in eosinophil count after 6 months of treatment. ALS is not typically associated with a systemic inflammatory response, and baseline eosinophil counts in the treated and placebo groups were  $0.129$  and  $0.127 \times 10^9/L$ , respectively. However, the eosinophil lowering effect of DEX was not diminished in patients ( $n=42$ ) with higher eosinophil counts (i.e.  $>0.25 \times 10^9/L$ ), where a 75% decrease was observed after 6 months of treatment. There were no clinically significant changes in monocytes and lymphocytes. Neutropenia ( $ANC < 1.5 \times 10^9/L$ ) was observed in 29 DEX treated patients (6.1%) and 8 (1.7%) patients receiving placebo, and was reversible upon withdrawal of treatment. DEX was well-tolerated in Phase II and III trials in ALS subjects.

**Conclusions:** Clinical experience in over 500 DEX-treated ALS patients demonstrates that DEX produces a slowly-developing, highly-significant persistent eosinophil reduction in humans. As DEX is well-tolerated in humans following exposures up to 18 months, it may represent a novel therapeutic approach for the treatment of eosinophil-associated disorders.

## POSTER 12

**MICRORNAS CONTROL TRANSCRIPTIONAL NETWORKS THAT REGULATE EOSINOPHIL DIFFERENTIATION.**

Ming Yang<sup>1</sup>, Fiona Evers<sup>1</sup>, Ian G. Young<sup>2</sup> and Paul S. Foster<sup>1</sup>

<sup>1</sup>Priority Research Centre for Asthma and Respiratory Disease, Department of Microbiology and Immunology, School of Pharmacy and Biomedical Sciences, Faculty of Health and Hunter Medical Research Institute, University of Newcastle, Newcastle, Australia

<sup>2</sup>Department of Molecular Biosciences, The John Curtin School of Medical Research, Australian National University, Canberra, Australia

**Background:** MicroRNA (miRNAs) are small non-coding RNAs of approximately 22 nucleotides in length, which act as endogenous sequence-specific inhibitors of protein translation and are highly conserved across species\*. It is now recognized that miRNA can regulate complex transcriptional networks underpinning immunity and diseases such as cancer, heart disease and respiratory inflammatory disorders. These molecules also play important roles in the regulation of growth and differentiation of leukocytes. In this study we will investigate the role of miRNA in the regulation of eosinophil differentiation.

**Methods:** Bone marrow cells from BALB/c WT mice were cultured under eosinophil differentiation conditions for 14 days and were then identified by both flow cytometry (CD11b+CD11c-SiglecF+ cells as eosinophils) and Giemsa staining. Expression levels of miRNAs were determined 1 hour before the addition of IL-5 (day 4) and on days 6, 8, 10, 12 and 14 in the presence of IL-5. The putative mRNA targets -of the miRNAs with  $\geq 5$  fold changes- were predicted by Genespring software. Furthermore, quantitative PCR was employed to verify the miRNA Array and to confirm the expression levels of putative mRNA targets, at the above timepoints. The interplay between miRNAs and potential target mRNAs was analyzed by cross-comparison with TargetScan, MeSH database, miRanda and IPA software.

**Results:** 68 miRNAs were identified during eosinophil development *in vitro*. 8 miRNAs were selected to verify the microRNA array data. 348 potential target mRNAs were identified, using TargetScan and Mesh database. The top 10 canonical pathways, involved in eosinophil differentiation, were found to be hypothetically regulated by many of these target mRNAs. Furthermore, 13 miRNAs were predicted to target CCR3 or IL-5Raplha, and 7 miRNAs for GATA1 or PU.1 (transcription factors), respectively. These molecules critically regulate the development of eosinophils. Importantly, we have also identified 17 miRNAs that target TLR-4 or TLR-13, suggesting that these miRNAs may regulate host defense mechanisms in eosinophils.

**Discussion:** We demonstrate that there are specific miRNA networks that may regulate various aspects of eosinophil differentiation. Specific miRNA sets are used to regulate the expression of CCR3, IL-5Raplha and transcription factors GATA1 and PU.1. There appears to be a dynamic interplay between miRNA networks and the control of the expression of various TLRs. We have established the miRNA networks that are associated with the regulation of different aspects of eosinophil differentiation and identify their putative targets, which may be useful for the therapeutic modulation of eosinophil-driven allergic diseases.

\*Foster PS, Plank M, Collison A, et al. (2013). The emerging role of microRNAs in regulating immune and inflammatory responses in the lung. *Immunological Reviews*. 253:198-215.

## POSTER 13

## DO EOSINOPHILS HAVE A ROLE IN GRAFT-VERSUS-HOST DISEASE?

Christine Wennerås<sup>1,2</sup>, Julia Cromvik<sup>2</sup>, Marianne Johansson<sup>1</sup>, Krista Vaht<sup>2</sup>, Jan-Erik-Johansson<sup>2</sup>.

Departments of Infectious Diseases<sup>1</sup> and of Hematology and Coagulation<sup>2</sup>, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden

**Background:** Eosinophilia and eosinophilic tissue infiltration have been observed in patients with acute and chronic graft-versus-host disease (GVHD). It is unknown if eosinophils are involved in GVHD or are mere bystander cells.

**Methods:** Adult hematopoietic stem cell transplant recipients (n=37) with and without GVHD, treated and untreated with systemic corticosteroids, were investigated with respect to absolute and relative eosinophil counts and levels of 14 surface markers by 4-color flow cytometry. Multivariate analysis of pattern recognition (Partial Least Squares Projections to Latent Structures-Discriminant Analysis) was used to see if the eosinophilic data could segregate the various patient subgroups.

**Results:** Multivariate analysis revealed that blood eosinophils from patients with acute GVHD had relatively higher levels of CD69, CD23, CD54, CD49d, CD11c and formyl peptide receptors, and were more numerous in the circulation compared to what was seen in patients without GVHD. This suggested that eosinophils were activated in acute GVHD. Eosinophils from patients with chronic GVHD also appeared to be activated compared to eosinophils from patients with no GVHD: their eosinophils had upregulated CD69, CRTH2, CD11c, CD16, CD9, CD18, formyl peptide receptors and CD49d relative to eosinophils in patients lacking GVHD. When comparing eosinophils from patients with the two forms of GVHD, it was seen that CD49d and CD23 were more associated with acute GVHD, whereas CD18, CD11c and CRTH2 were more associated with chronic GVHD. Finally, systemic corticosteroid therapy drastically altered the phenotype of eosinophils in patients with GVHD, but not in those without GVHD, an indirect indication that eosinophils were activated in GVHD, and hence susceptible to the anti-inflammatory effects of corticosteroids.

**Conclusions:** Eosinophils are likely to play a role in GVHD since they display an activated phenotype. Moreover, the different phenotypes exhibited by eosinophils in acute and chronic GVHD suggest that blood eosinophils receive different signals of activation from the tissues in these two forms of GVHD. Possibly, eosinophilic phenotypes could be used to facilitate the diagnosis of GVHD.

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## POSTER 14

**EOSINOPHILS CONTROL THE RESOLUTION OF INFLAMMATION THROUGH THE 12/15-LIPOXYGENASE PATHWAY**Makoto Arita<sup>1</sup><sup>1</sup>Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo 113-0033, Japan

**Background:** Inflammation is a defensive response to injury and infection, but inflammation must be resolved in a timely fashion to avoid local tissue damage that can contribute to a range of chronic diseases. Recently, we showed that eosinophils express a high level of 12/15-lipoxygenase (12/15-LOX) and are the major cell type producing 12/15-LOX-derived mediators locally in the resolution phase. *In vivo* depletion of eosinophils caused a resolution deficit, and adoptive transfer of wild-type eosinophils, but not 12/15-LOX-deficient eosinophils, rescued the resolution deficit in eosinophil-depleted mice (1). These results indicate that eosinophils are recruited to the inflamed loci during the resolution phase, where they locally produce pro-resolving mediators via the 12/15-LOX-initiated biosynthetic route.

**Objectives:** To understand the molecular mechanism by which eosinophils promote resolution of inflammation in a 12/15-LOX-dependent manner.

**Methods:** For acute peritonitis, male C57BL/6 mice were given i.p. injection of zymosan A (1mg). At the indicated time points, peritoneal exudates and/or draining lymph nodes were collected. For determination of cellular composition, cells were stained with fluorescent labeled antibodies and were analyzed for composition with a fluorescence-activated cell sorter (FACS) using FACSCalibur. To assess whether eosinophils regulate the macrophage phenotype in the resolution phase, we examined gene expression in macrophages at 24 h after zymosan challenge using the Affymetrix mouse 430A, Version 2.0 arrays. A number of differentially regulated genes in  $\Delta$ dblGATA mice were identified. Also, to identify genes regulated by eosinophils in a 12/15-LOX dependent manner. Wild-type or 12/15-LOX-deficient eosinophils isolated from IL-5 transgenic mice were adoptively transferred into  $\Delta$ dblGATA mice and gene expression in macrophages was monitored. Eosinophils were isolated from peritoneal lavages of IL-5 transgenic mice (kindly provided by Dr. Kiyoshi Takatsu). 12/15-LOX deficient eosinophils were isolated from 12/15-LOX deficient mice crossed with IL-5 transgenic mice.

**Results:** We demonstrate that eosinophils induce the expression of a chemokine in macrophages in the resolution phase by 12/15-LOX-dependent mechanisms, and this pathway regulates the migration of phagocytes carrying antigens to draining lymph node (DLN) and the clearance of neutrophils from the inflammatory site. We also demonstrate that eosinophils are important regulator for inflamed DLN remodeling in the resolution phase, and that this process is also regulated by 12/15-LOX.

**Conclusions:** These results demonstrate that eosinophils have an unexpected key role in the resolution of inflammation and inflamed DLN remodeling through the 12/15-LOX pathway.

**References:** (1) Yamada T, Tani Y, Nakanishi H, Taguchi R, Arita M, Arai H. *FASEB J* 25, 561-568 (2011)

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## POSTER 15

**THE ASSOCIATION OF EOSINOPHILS AND THEIR CATIONIC PROTEINS TO CANCER AND CANCER DEVELOPMENT**

Kristin Blom<sup>1</sup>, Jenny Rubin<sup>1</sup>, Lena Håkansson<sup>1</sup>, Marie Carlson<sup>2</sup>, Peter Nygren<sup>3</sup> and Per Venge<sup>1</sup>.

<sup>1</sup>Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden, <sup>2</sup>Department of Medical Sciences, Gastroenterology Research Group, Uppsala University, Uppsala, Sweden and <sup>3</sup> Department of Radiology, Oncology and Radiation Science, Section of Oncology, Uppsala University, Uppsala, Sweden.

**Background:** A role of eosinophil granulocytes in cancer and cancer development has been suggested but is still somewhat enigmatic. Infiltration of eosinophils as well as elevated levels of the eosinophil cationic proteins, eosinophil cationic protein (ECP) and eosinophil derived neurotoxin / eosinophil protein x (EDN/EPX), have previously been shown in malignant tumours such as colorectal and renal cancer and different forms of leukaemia. The association between eosinophils and poor outcome in Hodgkin's lymphoma was also indicated.

**Objective:** To study the role of eosinophil granulocytes in human cancer, with focus on the association of EDN/EPX and ECP in a genetic and phenotypic manner. We hypothesize that the eosinophil granulocyte plays important roles in some types of cancer, and that the genetically determined alterations in activity and production of EDN/EPX and ECP partly may determine this role.

**Methods:** Histochemically stained preparations of cells from several different malignant solid tumours were analysed by microscopic examination. DNA was extracted from whole blood and frozen tissue from 226 patients with colon and rectal cancers and 300 healthy subjects. A diagnosis of cancer was based on standardized criteria. Some of the patients also had previously been diagnosed with ulcerative colitis. The EDN/EPX405 (G>C, rs2013109), ECP434 (G>C, rs2073342) and ECP562 (G>C, rs2233860) gene polymorphisms were analysed, by the 5' nuclease allelic discrimination assay. The patient tumour cells were also analysed by flow cytometry.

**Results:** Eosinophils were observed in colorectal cancer, ovarian cancer, and kidney cancer at a ratio of 0 to 7 %. The presence of CD45+ leukocytes varied between 15 to 35 % and the presence of CD16+ granulocytes between 3 and 9 %.

In patients with cancer/dysplasia and ulcerative colitis (n=27) carrying the ECP434-GC/CC or ECP562-GC/CC genotypes the relative risks to develop dysplasia/cancer were 2.4 (95% CI, 1.1-5.2) for ECP434 and 2.8 (95% CI, 1.3-5.8) for ECP562 as compared to healthy individuals.

**Conclusion:** The present on-going study has identified the presence of granulocytes and eosinophils in several malignant cancers. An association between polymorphisms in the EDN/EPX and ECP genes and the development of dysplasia/cancer in patients with ulcerative colitis was shown. These novel findings suggest essential roles of eosinophils and their cationic proteins ECP and EDN/EPX in the disease processes of different types of malignant cancers.

## POSTER 16

**MOUSE INTESTINAL EOSINOPHILS HAVE AN ANTIGEN PRESENTING CELL PHENOTYPE AND ACQUIRE LUMENAL ANTIGEN**

Kalmia S. Buels<sup>1</sup>, Jason J. Xenakis<sup>1</sup>, Admira Dinaj<sup>1</sup>, and Lisa A. Spencer<sup>1</sup>

<sup>1</sup>Division of Allergy and Inflammation, Beth Israel Deaconess Medical Center, Boston, MA USA

**Background:** Eosinophils are found in the small intestine of healthy individuals and intestinal eosinophil numbers increase in food allergy. Eosinophils recovered from sites of allergic inflammation have previously been shown to express MHC class II (MHCII) and costimulatory molecules required for antigen presentation. In addition, *in vitro* experiments in humans and mice and *in vivo* experiments in mice have established that eosinophils are capable of antigen presentation. We asked whether small intestine eosinophils could be involved in antigen uptake and presentation in naive and sensitized mice.

**Methods:** Flow cytometry was used to measure expression of MHCII, CD80 and CD11c in eosinophils (LiveCD45<sup>+</sup>Siglec F<sup>hi</sup>SS-C<sup>hi</sup>) isolated from small intestines of BALB/c mice.

Cellular uptake of ovalbumin (OVA) antigen from the small intestine lumen of live mice (either naive or OVA/alum sensitized (50 mg OVA IP on days 0, 7, and 14)) was quantified by flow cytometry. Intestinal cells were isolated following injection of fluorescently-labeled OVA (20 mg/loop) into the lumen of loops created by tying off 5 cm segments of intestine in anesthetized mice, and antigen uptake in individual cells was determined relative to control mice challenged with PBS.

**Results:** Small intestine eosinophils in naive mice expressed cell surface MHCII, CD80 and CD11c. Eosinophils isolated from intestinal loops of naive mice 45 min after OVA antigen was injected into the lumen exhibited no evidence of OVA uptake, while  $13.2 \pm 2.6\%$  (mean  $\pm$  SD) of dendritic cells (CD11c<sup>hi</sup>Siglec F<sup>-</sup>) recovered were OVA<sup>+</sup>. In contrast,  $13.7 \pm 6.9\%$  of eosinophils isolated from intestinal loops of OVA-sensitized mice were OVA<sup>+</sup>, and  $18.9 \pm 5.8\%$  of dendritic cells were also OVA<sup>+</sup> in these mice.

**Conclusions:** Resident small intestine eosinophils express an antigen presentation phenotype in naive mice. Our intestinal loop data further show small intestine eosinophils of antigen-primed mice acquire lumen-derived antigen *in situ* and suggest eosinophils may impact intestinal immunity via presentation of orally ingested antigen in previously sensitized individuals.

**Grant support:** This work was funded by the following grants to LAS: American Partnership for Eosinophilic Disorders (APFED) Hope Pilot Grant, William F. Milton Fund, and NIH 3P30DK034854 and R01HL095699

## POSTER 17

**DIFFERENTIAL EOSINOPHIL ACTIVATION IN CHRONIC AND ACUTE GRAFT VERSUS HOST DISEASE MAY DEPEND ON ALTERED CYTOKINE RESPONSES**Jennie Andersson<sup>1</sup>, Julia Cromvik<sup>1</sup> and Christine Wennerås<sup>1,2</sup><sup>1</sup>Department of Infectious Diseases, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; <sup>2</sup>Department of Hematology and Coagulation, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

**Background:** We have previously observed that blood eosinophils are differentially activated in acute and chronic Graft Versus Host Disease (GVHD). In this study we wanted to examine cytokines of importance for the maturation and release of eosinophils from the bone marrow as well as chemokines implicated in eosinophil migration into tissues, during GVHD.

**Methods:** Plasma was collected from hematopoietic transplant recipients diagnosed with either chronic or acute GVHD. Both corticosteroid-treated and untreated patients were studied. Plasma was also collected from transplant recipients not suffering from GVHD. The plasma samples were analyzed using multiplex assays, based on Luminex technology, in order to determine the levels of IL-1 $\beta$ , IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF) basic, granulocyte colony stimulating factor (G-CSF), granulocytemacrophage colony stimulating factor (GM-CSF), interferon gamma (IFN- $\gamma$ ), IFN- $\gamma$  inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1 $\alpha$ , MIP-1 $\beta$ , platelet derived growth factor (PDGF)-BB, regulated upon activation normal T cell expressed and secreted (RANTES), tumor necrosis factor (TNF)- $\alpha$  and vascular endothelial growth factor (VEGF). Cytokine data was analyzed using the multivariate method "orthogonal projections to latent structures," to obtain variables of importance (VIP) for distinguishing transplant recipients with acute, chronic and no GVHD, respectively.

**Results:** IL-17, FGF basic and IL-8 were the three cytokines with the highest VIP values, when comparing untreated patients suffering from acute GVHD to untreated patients with chronic GVHD. The three cytokines that showed the highest level of discriminatory power when studying patients with chronic GVHD, before and after corticosteroid treatment, were IL-4, IL-7 and IFN- $\gamma$ . Further, IP-10 and eotaxin presented the highest VIP values when comparing patients with acute GVHD before and after corticosteroid treatment. In addition, IL2, IL-4 and IL-7 proved to be the most discriminatory cytokines when comparing corticosteroid-treated patients experiencing acute GVHD to corticosteroid-treated patients suffering from chronic GVHD. Comparing untreated transplant recipients with chronic GVHD to transplant recipients without GVHD revealed IL-4, TNF- $\alpha$ , IL-1ra and IFN- $\gamma$  as discriminators.

**Conclusions:** IL-2, IL-4, IL-8, IL-17, IFN- $\gamma$ , IP-10, TNF- $\alpha$  and eotaxin are all involved in eosinophil activity and homeostasis. The different profiles that emerge for acute and chronic GVHD with regards to these cytokines may be one contributory factor to the differential activation states of eosinophils previously observed in GVHD patients.

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## POSTER 18

## INDUCTION OF MALIGNANT PLASMA CELL PROLIFERATION BY EOSINOPHILS

Tina W. Wong<sup>1</sup>, Hirohito Kita<sup>2</sup>, Curtis A. Hanson<sup>3</sup>, Denise K. Walters<sup>1</sup>, Bonnie K. Arendt<sup>1</sup>, Diane F. Jelinek<sup>1,2</sup>

<sup>1</sup>Department of Immunology, Mayo Clinic, Rochester, MN; <sup>2</sup>Department of Internal Medicine, Mayo Clinic, Rochester, MN;

<sup>3</sup>Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905

**Background:** Given the recently described role for mouse eosinophils (Eos) in the regulation of normal bone marrow (BM) plasma cell (PC) homeostasis, we questioned whether in the human PC malignancy, multiple myeloma (MM), Eos may contribute to disease progression by influencing tumor cell survival and/or proliferation.

**Methods:** BM biopsies from MM patients and healthy donors were subjected to H&E and immunofluorescence staining to assess the localization of Eos and PCs. *In vitro* chemotaxis assays were performed using previously characterized human myeloma cell lines and Eos isolated from human BM aspirates to determine the mechanism of Eos and PC colocalization in the BM. <sup>3</sup>H-thymidine-incorporation was used to assess MM cell line proliferation in co-culture with Eos and/or BM stromal cells (SCs). Proliferation of purified CD138+ primary MM cells was assessed using BrdU-labeling. Contact-dependency of the Eos-induced MM proliferation was evaluated using transwell co-cultures or via treatment of MM cells with Eos culture supernatant. Eos culture supernatants were fractionated by ultracentrifugation for isolation of microparticles in order to determine the contributions of microparticles to the induction of MM proliferation by Eos. Neutralization of IL-6 was performed to assess the dependency of Eos-induced MM cell proliferation on IL-6.

**Results:** Histological studies demonstrated that PCs and Eos can be found in close proximity in the human BM. Chemotaxis studies suggested that this colocalization may reflect migration of both cell types to chemokines secreted by BM SCs, which can include, but is not solely dependent upon, CXCL12. In co-culture, Eos isolated from either BM or peripheral blood were shown to induce proliferation of 3 out of 6 MM cell lines. Mechanistically, the induced proliferation was largely mediated through a soluble factor(s) secreted by Eos and is not driven by Eos-derived microparticles. Similarly, proliferation of primary CD138+ MM cells was enhanced when treated with Eos culture supernatant. Using a multicellular *in vitro* system, we showed that SCs and Eos have non-redundant roles in their support of MM cell growth. Whereas SCs induced MM cell proliferation predominantly through the secretion of IL-6, Eos stimulated growth of these malignant cells via an IL-6-independent mechanism.

**Conclusions:** Our data show that Eos and MM cells can colocalize in the BM via their coordinated migration toward BM SCs to create a niche that promotes tumor cell growth. Furthermore, our study demonstrates for the first time a role for Eos in the pathology of MM by the IL-6-independent induction of malignant cell proliferation.

**Grant support:** This work is supported by the Mayo Foundation and NIH Pre-doctoral Immunology Training Grant T32 AI07425.

## POSTER 19

## IMPLICATION OF INNATE IMMUNE RECEPTORS IN EOSINOPHIL TUMORICIDAL ACTIVITY

Solène Gatault<sup>1</sup>, Caroline Stremnitzer<sup>2</sup>, Marie Delbeke<sup>1</sup>, Fanny Legrand<sup>1\*</sup>, Jean-Emmanuel Kahn<sup>3,4</sup>, Guillaume Lefèvre<sup>3,5</sup>, Erika Jensen-Jarolim<sup>2,6</sup>, Monique Capron<sup>1</sup>

<sup>1</sup>U995 Inserm, University Lille <sup>2</sup> Medical School, Lille, France

<sup>2</sup>Department of Pathophysiology and Allergy Research, Division I – Comparative Immunology and Oncology, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

<sup>3</sup> French National Eosinophilic Network, Lille, France

<sup>4</sup>University Versailles-Saint Quentin en Yvelines, Department of Internal Medicine, Hospital Foch, Suresnes, France

<sup>5</sup>Immunology Institute, EA<sup>2686</sup>; Department of Internal Medicine and Clinical Immunology, Regional University Hospital, Lille, France

<sup>6</sup>Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria

\*Present address: Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

**Background:** Eosinophils may have an important anti-tumor role, notably on colorectal cancer. Although eosinophils exhibit cytotoxic effects towards a colorectal carcinoma cell line (Colo-205), immune-receptors involved in this mechanism remain to be analyzed.

**Methods:** Peripheral blood eosinophils were purified from allergic and non-allergic donors by immunomagnetic negative selection. After co-incubation of eosinophils and Colo-205 cells, percentage of tumor cell apoptosis was measured by flow cytometry at several timepoints. The presence of immune receptors, such as TLR4 and NLRP3, was studied by flow cytometry and RT-PCR. Cytokine and mediator release was determined in co-culture supernatants by ELISA. The implication of the different receptors was analyzed by using agonists or neutralizing antibodies.

**Results:** Eosinophils exerted varying tumoricidal properties depending on the allergic status of donors. Our data showed that human eosinophils from allergic donors induced significantly more Colo-205 apoptosis than eosinophils from non-allergic donors ( $P < 0.05$ ). Stimulation of eosinophils from allergic donors with matched allergens (e.g. Derp 2, NA-STM-1) had no influence on-tumor cell cytotoxicity.

A thirty minute-prestimulation of eosinophils with LPS, a Der p 2 co-factor, induced a significant decrease of Colo-205 apoptosis induced by eosinophils in allergic donors only ( $P < 0.05$ ). TLR4, the immune receptor of LPS, was present in the membrane of eosinophils. The neutralization of TLR4 did not restore the cytotoxic effects of eosinophils.

RT-PCR and flow cytometry approaches revealed that human eosinophils express NLRP3 which is an intracellular receptor and a component of the inflammasome. NLRP3 polymorphisms are associated with allergy and it has also been reported to negatively regulate colon tumorigenesis. NLRP3 induces the release of IL-18, cytokine involved in protection against colorectal tumor. IL-18 is released by eosinophils incubated with Colo-205 whereas neutralizing anti-human IL-18 inhibited Colo-205 cell death ( $P < 0.05$ ). This anti-tumor effect of NLRP3 is only due to eosinophils because it was not expressed by Colo-205. These results provide a new mechanism for eosinophils in the anti-tumor cytotoxicity.

**Conclusion:** Our findings suggest that activation of different immune responses influences the cytotoxic effect of human eosinophils. Further investigations might be important for the better understanding of the mechanisms implicated in their anti-tumor role.

## POSTER 20

**USING THE ALLERGIC IMMUNE SYSTEM TO TARGET CANCER: ACTIVITY OF IGE ANTIBODIES SPECIFIC FOR HUMAN CD20 AND MUC1.**

Pearline Z. Teo<sup>1,2</sup>, Paul J. Utz<sup>2</sup>, Joseph A. Mollick<sup>2,3</sup>

<sup>1</sup>Molecular Engineering Lab, Agency for Science, Technology and Research, Singapore 138673, Singapore

<sup>2</sup>Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

<sup>3</sup>Division of Oncology, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA

Monoclonal antibodies are widely used in the treatment of many B cell lymphomas and certain solid tumors. All currently approved therapeutic monoclonal antibodies are of the immunoglobulin G (IgG) isotype. We hypothesized that tumor-specific monoclonal antibodies of the IgE isotype may serve as effective cancer therapeutics. To test this hypothesis, we produced mouse-human chimeric IgE antibodies specific for the human B cell antigen CD20 and the epithelial antigen MUC1. We demonstrate here that anti-hCD20 IgE antibodies have *in vitro* cytotoxic activity when used with purified allergic effector cells derived from umbilical cord blood. At an effector-tumor ratio of 2:1, mast cells and tumor-specific IgE induced a 2.5-fold increase in tumor cell death, as compared to control IgE. Similar results were observed when eosinophils were used as effector cells. In an *in vivo* murine model of breast carcinoma, administration of anti-hMUC1 IgE reduced the growth of MUC1(+) tumors by 25-30 % in hFcεRI transgenic mice. In contrast, local production of IgE and cytokines chemotactic for macrophages, eosinophils and mast cells led to complete tumor eradication. These results suggest that allergic effector cells activated by IgE and cell surface antigens have the capacity to induce tumor cell death *in vitro* and *in vivo*. The use of chimeric antibodies and hFcεRI transgenic mice will greatly enhance investigations in the nascent field of allergo-oncology.

## POSTER 21

**DEVELOPMENT OF A SUSPENSION ARRAY ASSAY IN MULTIPLEX TO SIMULTANEOUSLY MEASURE SERUM LEVELS OF EOSINOPHIL GRANULE PROTEINS**

Michelle A. Makiya<sup>1</sup>, Jessica A. Christensen<sup>2</sup>, Thomas B. Nutman<sup>2</sup> and Amy D. Klion<sup>1</sup>

<sup>1</sup>Eosinophil Pathology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

<sup>2</sup>Helminth Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

**Background:** Serum levels of major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO) have been shown to correlate with disease severity in a wide variety of eosinophilic disorders. Whereas radioimmunoassay, immunofluorescence and ELISA have been used to measure eosinophil granule proteins, none of these methods efficiently measure MBP, ECP, EDN and EPO simultaneously.

**Methods:** Mouse monoclonal antibodies against MBP, ECP, EDN and EPO were coupled to carboxylated magnetic microspheres via two-step carbodiimide reaction. After confirmation of antibody coupling and coupling repeatability, the multiplex assay was optimized. A panel of 32 subject serum samples was screened using purified eosinophil granule protein controls for development of a serum standard control. The eosinophil granule protein multiplex detection method was validated by comparison of eosinophil granule protein levels in serum from 25 eosinophilic subjects with an established ELISA for MBP, ECP and EDN. Additional testing was performed in serum from 42 eosinophilic subjects and 19 healthy donors.

**Results:** The serum standard control was found to be accurate at concentrations greater than 1.485 ng/ml, 2.149 ng/ml and 1.69 ng/ml for MBP, ECP and EPO, respectively. The serum standard curve for EDN was accurate within the range of 2.025 ng/ml and 28.599 ng/ml. Thus, serum samples were diluted 1:250 for accurate concentration calculations within the linear region of the standard curves. Comparison of serum from eosinophilic subjects analyzed by both ELISA and multiplex showed a significant correlation between the serum levels of MBP, ECP and EDN ( $r = 0.8569$ ;  $P < 0.0001$ ,  $r = 0.6356$ ;  $P = 0.0006$  and  $r = 0.8600$ ;  $P < 0.0001$ , respectively, Spearman rank correlation). Absolute eosinophil count was significantly correlated with the concentrations of MBP, EDN and EPO ( $r = 0.7279$ ,  $0.6499$ , and  $0.6720$  respectively;  $P < 0.0001$ ), but not with the concentration of ECP in serum from eosinophilic subjects. Similarly, serum levels of MBP ( $r = 0.3531$ ;  $P = 0.0218$ ), EDN ( $r = 0.3046$ ;  $P = 0.0498$ ) and EPO ( $r = 0.3764$ ;  $P = 0.0140$ ), but not ECP, were significantly correlated with CD69 expression on blood eosinophils.

**Conclusion:** A multiplex suspension array system was developed to simultaneously measure the concentration of MBP, ECP, EDN and EPO using minute quantities of serum. This assay will facilitate studies of individual granule proteins as biomarkers of disease activity and therapeutic efficacy in eosinophilic disorders.

**Grant support:** *This study was funded by the Division of Intramural Research, NIAID, NIH.*



## POSTER 22

**RHO AND RAC ARE ESSENTIAL FOR SECRETION OF EOSINOPHIL-ASSOCIATED RNASES FROM HUMAN AND MOUSE EOSINOPHILS**

Revital Shamri, Kristen M. Young and Peter F. Weller

Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

**Background:** Cytoplasmic secretory granules of eosinophils contain highly basic proteins. Among them are the eosinophil-associated RNases (EARs): the human eosinophil-derived neurotoxin and eosinophil cationic protein (ECP) and their murine orthologues. In the last decade several studies, both *in vitro* and *in vivo*, have revealed roles for eosinophils and their EARs in host defense and immunomodulation. Moreover, tissue deposits of ECP are associated with a range of inflammatory disorders, such as bronchial asthma and Crohn's disease, and ECP levels in body fluids are clinical markers for the diagnosis of chronic inflammatory diseases. Although critical roles of EARs and other granule proteins in host defense and in human pathology are known for decades, mechanisms that regulate secretion of eosinophil granule proteins are poorly understood, especially concerning the involvement of cytoskeletal machinery in the secretory process. In these studies, we focus on the involvement of Rho and Rac, the small G-proteins that play key roles in the regulation of cytoskeleton rearrangements as well as in cells division, survival, migration and adhesion. Our study aimed to understand the involvement of cytoskeletal elements, such as Rho and Rac, in EAR secretion.

**Methods:** Human and mouse eosinophils were pre-inhibited with specific blockers of Rho (RhoA, B and C), ROCK (Rho kinase) and Rac (Rac1, 2 and 3), and were stimulated with CCL11. The secretion of enzymatically active EARs of pre-inhibited and stimulated eosinophils was detected by using an RNase-activity assay. ELISA assays were utilized to measure secretion of human ECP.

**Results:** Inhibition of Rho activity blocked EAR secretion from human and mouse eosinophils in a dose response-dependent manner. However, Rho-dependent EAR secretion was found to be ROCK independent, since a ROCK inhibitor didn't block and even increased EAR secretion. Therefore, other Rho downstream effectors might be involved in this process, and ROCK might serve as a negative regulator of the secretion process. Additionally, a Rac inhibitor robustly blocked CCL11-mediated EAR secretion in mouse and human eosinophils. These results suggest that both Rho and Rac are involved in EAR secretion in human and mouse eosinophils, probably through their activity in cytoskeletal rearrangements. Although commonality in the involvement of Rho and Rac in EAR secretion in human and mouse eosinophils, the cytoskeletal rearrangements needed for EAR secretion were not identical in eosinophils from the two species. While EAR secretion in human eosinophils was blocked by actin depolymerization, in mouse eosinophils actin depolymerization tended to increase EAR secretion.

**Conclusions:** Our studies elucidate a platform to understand the cytoskeleton support required for the release of granule proteins from eosinophils, and emphasize commonality and differences in the secretion mechanisms utilized by human vs mouse eosinophils.

**Grant support:** *This work is funded by grants from NIH (RO1/R37 AI020241, RO1 AI05164).*

## POSTER 23

**THYMIC STROMAL LYMPHOPOIETIN STIMULATES THE FORMATION OF EOSINOPHIL EXTRACELLULAR TRAPS**

Mahbubul Morshed<sup>1,2</sup>, Shida Yousefi<sup>2</sup>, Christina Stöckle<sup>2</sup>, Hans-Uwe Simon<sup>2</sup>, Dagmar Simon<sup>1</sup>

<sup>1</sup>Department of Dermatology, Inselspital, Bern University Hospital, and <sup>2</sup>Institute of Pharmacology, University of Bern, Bern, Switzerland

**Background:** Thymic stromal lymphopoietin (TSLP) that is released by epithelial cells upon certain environmental triggers activates cells of the innate and adaptive immune system resulting in a preferential T helper 2 immune response. By releasing eosinophil extracellular traps (EETs), eosinophils achieve an efficient extracellular bacterial killing. EET release, however, has been observed both in infectious and non-infectious eosinophilic diseases. Here, we aim to investigate whether eosinophils generate functional EETs as a direct response to TSLP, and further to study the extra- and intra-cellular mechanisms involved in this process as well as TSLP receptor (TSLPR) expression by eosinophils *in vitro* and *in vivo*.

**Methods:** TSLPR expression on blood and tissue eosinophils was assessed by immunoblotting, flow cytometry and immunofluorescence staining. Purified eosinophils were stimulated with recombinant human TSLP. The release of extracellular DNA in association with eosinophilic cationic protein (ECP) was detected by fluorescence staining techniques and confocal microscopy. In addition, cell survival, cell adhesion, production of reactive oxygen species (ROS), and the inhibition of bacterial growth by TSLP-stimulated eosinophils were measured.

**Results:** TSLPR was observed on peripheral blood eosinophils as well as on tissue infiltrating eosinophils in skin diseases. TSLP did not affect eosinophil survival, but induced the formation of EETs consisting of mitochondrial DNA in association with ECP in a concentration- and time-dependent manner. EET release could be inhibited either by blocking cell adhesion or ROS production. While eosinophils prevented growth of both *Staphylococcus aureus* and *Staphylococcus epidermidis*, the latter were unable to elicit EET formation and eosinophils required additional TSLP stimulation to achieve this antibacterial activity.

**Conclusions:** TSLP directly stimulates eosinophils to produce EETs. Our observations link epithelial TSLP expression triggered by environmental factors with pathogen defense mechanisms involving eosinophils.

## POSTER 24

## ENZYMATIC ANALYSIS OF MOUSE EOSINOPHIL ASSOCIATED RIBONUCLEASE 11

Kelsey J. Yamada<sup>1</sup>, Katia E. Garcia-Crespo<sup>1</sup>, Kimberly D. Dyer<sup>1</sup>, James J. Lee<sup>2</sup>, and Helene F. Rosenberg<sup>1</sup>,

<sup>1</sup>Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA and <sup>2</sup>Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

**Background:** Mouse Eosinophil Associated Ribonucleases (mEARs) are a diverse family of orthologs of the eosinophil derived neurotoxin (EDN) and eosinophil cationic protein (ECP). In humans, EDN is a nominally cationic protein that has retained significant ribonuclease activity. However, evolutionary constraints have enhanced the cytotoxicity/cationicity of ECP at the expense of ribonuclease activity since gene duplication in primates. Mouse EARs are hypothesized to have undergone an unusual pattern of evolution called “rapid birth-death and gene sorting”, also documented in the T-cell receptor, immunoglobulin, and major-histocompatibility complex gene families. This rapidly expanded gene family consists of ~15 genes of which mEar-1, -2, -6/7, -5/11 have been positively identified within eosinophil secondary granules. Mouse EAR11 has retained the RNase A family characteristic sequence motifs and is also expressed by alternatively-activated macrophages the lung in response to Th2 cytokine stimuli.

**Methods:** cDNA encoding mEAR11 (GenBank no. AY015178) was cloned into a pET24a+ vector and used to express and re-fold 6XHis-tagged protein from inclusion bodies in *E.coli* BL-21 star. Purified protein was used in enzymatic assays to determine mEAR11 ribonuclease activity against purified yeast tRNA. A mEAR11-K36R mutant was constructed to determine whether the mEAR11 catalytic lysine was essential to enzymatic function. Recombinant mEAR11-6xHis was also cloned into pPink $\alpha$ -HC vector and purified with Ni-NTA agarose beads after secretion into media during methanol induction of transfected *Pichia pastoris* cultures.

**Results:** Purified bacterial proteins exhibit gel electrophoretic mobility consistent with molecular mass of ~16 kDa, and kinetic constants obtained were  $K_m = 0.986$  mM,  $V_{max} = 3.43 \cdot 10^6$  mM / min,  $K_{cat} = 1.28$  s<sup>-1</sup>,  $K_{cat} / K_m = 1300$  s<sup>-1</sup>M<sup>-1</sup>. Purified recombinant yeast proteins are heterologous, with electrophoretic mobilities suggesting molecular masses ranging from 17-22 kDa.

**Conclusion:** We conclude that mEAR11 has prominent ribonucleolytic activity against the yeast tRNA substrate. We will compare the enzymatic activities of recombinant bacterial mEARs 1, 2, and 11 K36R. We will also determine the degree to which mEAR11 activity is susceptible to inhibition with purified ribonuclease inhibitor (RI). We will also determine whether endotoxin-free recombinant yeast mEAR11 can elicit leukocyte recruitment similar to that described by Yang and colleagues (Blood 2003) for mEar 2 and may ultimately generate an mEar 11 gene-deleted mouse. These results may offer further insight into the role of mEAR11 in allergic disease and respiratory infection.

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## POSTER 25

**IL-5 FAMILY CYTOKINES AND EOTAXIN POLARIZE SUSPENDED EOSINOPHILS TO FORM THE NUCLEOPOD, A UNIQUE UROPOD CONTAINING THE NUCLEUS**Shih-Tsung Han<sup>1,3,4</sup>, Deane F. Mosher<sup>1,2</sup>

<sup>1</sup>Departments of Biomolecular Chemistry and <sup>2</sup>Medicine, University of Wisconsin, Madison, Wisconsin, USA. <sup>3</sup>Department of Emergency Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC. <sup>4</sup>College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC.

**Background:** The shape change induced by IL-5 family cytokines or eotaxin in suspended eosinophils has not been characterized in depth.

**Methods:** Suspended eosinophils or neutrophils were examined in parallel by flow cytometry and confocal microscopy. Cells were stained by immunofluorescence with antibodies to PSGL-1, CD44, integrin  $\alpha M\beta 2$ ,  $\alpha$ -tubulin, and  $\gamma$ -tubulin, TRITC-phalloidin staining for filamentous actin, and DAPI for nucleus.

**Results:** Microscopy revealed that IL-5-induced rapid ( $\leq 5$  min) change in forward scatter correlates with eosinophil polarization, which includes the formation of a granular compartment and an agranular uropod enriched with PSGL-1, CD44, and activated  $\alpha M\beta 2$ . The nucleus was confined to the uropod. IL-3, GM-CSF, and eotaxin polarized eosinophils in the same way. Upon IL-5 stimulation, cortical distribution of filamentous actin was lost and replaced with abundant filamentous actin in the granular compartment. Cytochalasin B inhibited IL-5-induced polarization. The microtubule network was deformed after IL-5 treatment with part caging the nucleus in the uropod, and the microtubule organizing center was located precisely between the nucleus and granular compartment. Nocodazole inhibited IL-5-induced nucleus translocation to the uropod whereas taxol had no effect. In contrast to GM-CSF-treated eosinophils, GMCSF-treated neutrophils formed uropods that were enriched in PSGL-1 but the neutrophil nucleus was not confined to the uropod.

**Conclusions:** IL-5 family cytokines and eotaxin polarize suspended eosinophils to form a uropod containing the nucleus, which is distinctive from neutrophil uropod and we suggest be named the nucleopod.

**Grant supports:** NIH grants PO1 HL088594 and UL1 RR025011, CGMH fellowship

## POSTER 26

**CLUSTERING OF IL-5 FAMILY CYTOKINE RECEPTORS ON EOSINOPHIL NUCLEOPOD AND THE ASSOCIATION BETWEEN EOSINOPHIL PRIMING AND POLARIZATION**Shih-Tsung Han<sup>1,3,4</sup>, Deane F. Mosher<sup>1,2</sup>

<sup>1</sup>Departments of Biomolecular Chemistry and <sup>2</sup>Medicine, University of Wisconsin, Madison, Wisconsin, USA. <sup>3</sup>Department of Emergency Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC. <sup>4</sup>College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC.

**Background:** The distribution of IL-5 family cytokine receptors on primary eosinophils and the relationship between eosinophil polarization and IL-5 priming are unclear.

**Methods:** Suspended eosinophils with or without treatment were stained for subunits of IL-5 family cytokine receptors and downstream signaling molecules, and examined by confocal microscopy.

**Results:** IL-5R $\alpha$ , IL-3R $\alpha$ , or GM-CSFR $\alpha$ , which were scattered on the surface of unstimulated eosinophils, became clustered at the nucleopod upon specific stimulation with IL-5, IL-3, or GM-CSF, respectively. In contrast,  $\beta$ c became clustered at the nucleopod upon stimulation by any of IL-5 family cytokines. IL-5 treatment for 10 min resulted in the appearance of pJAK2, pSTAT1, pSTAT5, and pERK in the nucleopod in immediate proximity to the clustered receptors and induced nuclear translocation of pSTAT1, pSTAT5, and pCREB. IL-5-induced ERK phosphorylation at the nucleopod and granular compartment (granulomere) disappeared by 60 min, but unactivated ERK remained at these sites. Treatment with fMLF at 60 min resulted in much more robust ERK phosphorylation at the nucleopod and granulomere when compared to nonpolarized eosinophils not pretreated with IL-5.

**Conclusions:** IL-5 induces a stereotypical program of polarization in suspended human eosinophils that includes gathering of cytokine receptors and signaling molecules in the nucleopod and primes eosinophils to react more strongly to subsequent fMLF.

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## POSTER 27

## IL-33 STIMULATES IL-25 RELEASE BY HUMAN BLOOD EOSINOPHILS

Fanny Legrand<sup>1,4</sup>, Solène Gatault<sup>1</sup>, Marie Delbèke<sup>1</sup>, Claire Szymanski<sup>1,3</sup>, Geoffrey Mortuaire<sup>3</sup>, Sylvie Loiseau<sup>1</sup>, Michelle A. Makya<sup>4</sup>, Lionel Prin<sup>2,5</sup>, Jean Emmanuel Kahn<sup>2,6</sup>, Amy D. Klion<sup>4</sup> and Monique Capron<sup>1</sup>

<sup>1</sup>U995 Inserm, Université Lille-Nord de France, Faculté de Médecine, Lille, France

<sup>2</sup>French National Eosinophilic Network, Lille, France

<sup>3</sup>Otorhinolaryngology Department, University Hospital, Lille, France

<sup>4</sup>Eosinophil Pathology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases

<sup>5</sup>Université Lille-Nord de France, EA2686, Laboratoire d'Immunologie, Lille, France

<sup>6</sup>Université Versailles-Saint Quentin en Yvelines, Hôpital Foch, Service de Médecine Interne, Suresnes, France.

**Background:** Eosinophils recruited to epithelial barriers, in response to allergens or infectious agents, are associated with exacerbation of Th2-mediated inflammation. Mouse models have established that the 'innate-like Th2 cytokine', IL-25, which can be released by eosinophils, plays a major role in the initiation and maintenance of allergic inflammation. Furthermore, serum IL-25 levels were correlated with eosinophilia and clinical severity in patients with Churg-Strauss Syndrome. To date, only IL-3, GM-CSF and IL-5 have been shown to induce IL-25 release by eosinophils *in vitro*. Although IL-33 is also an 'innate-like Th2 cytokine' and is known to activate eosinophils through its receptor ST2, its ability to stimulate IL-25 release by eosinophils has not been explored.

**Methods:** Human peripheral blood eosinophils from healthy volunteers and eosinophilic patients were purified using negative selection (Miltenyi, MACS system). Tissue eosinophils were purified from nasal polyps (NP) of patients suffering from chronic rhinosinusitis using a published protocol optimized for enzymatic and mechanical digestion steps and cocktail antibodies for magnetic negative selection. Eosinophil purity was >95% for blood samples and 75-90% for NP. Eosinophil expression of ST2 was assessed by real time quantitative PCR and surface flow cytometry. Surface expression of CD69 was used as a marker of eosinophil activation. IL-33 and IL-25 mediated activation of eosinophils was further studied using different approaches: 1) measurement of Reactive Oxygen Species (ROS) by chemiluminescence, 2) assessment of eosinophil specific granule protein (EPO, ECP, EDN) release by ELISA, and 3) quantification of eosinophil chemotaxis using Boyden chambers. IL-25 production and release during eosinophil activation by IL-33 was studied by PCR, intracellular flow and measurement of IL-25 in cell supernatants.

**Results:** Flow cytometric analysis revealed membrane expression of ST2 receptor on blood (n=10/21) and not in tissue eosinophils (n=5). Both membrane and soluble ST2 receptor expression were up-regulated by IL-33 stimulation (n=5). Despite the lack of surface CD69 upregulation by IL-33, IL-33 induced strong and reproducible activation of eosinophils as indicated by release of ROS, ECP and EDN. Interestingly, although overnight stimulation with IL-33 (1-10-100ng/ml) induced dose-dependent release of IL-25 by eosinophils from normal donors (n=10, P<0.01), IL-25 was only detected in eosinophil supernatants from eosinophilic donors at the highest concentration (100 ng/ml) of IL-33 (n=11, P<0.05). IL-25 transcripts could not be detected in eosinophils, suggesting storage of IL-25 as a preformed cytokine (n=20). IL-33 induced chemotaxis in eosinophils from normal donors (n=10, P<0.01), but not from eosinophilic donors (n=10).

**Conclusions:** These data not only confirm that IL-33 can activate human eosinophils, but demonstrate that this activation induces the release of IL-25. Furthermore, the effect of IL-33 is more pronounced in normal donors, where it also exerts chemo-attractant properties. These results highlight a new connection between eosinophils and the two 'innate-like Th2 cytokines', IL-25 and IL-33. The relevance of these *in vitro* findings to the interplay between eosinophils, activated epithelia and Th2 cells in the context of a Th2 inflammatory response remains to be elucidated.

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## POSTER 28

**NOTCH SIGNALING MEDIATES GM-CSF-PRIMED HUMAN EOSINOPHIL TRANSENDOTHELIAL MIGRATION THROUGH A NON-CANONICAL SIGNALING PATHWAY IMPACTING ERK PHOSPHORYLATION**

Linying Liu, Haibin Wang, Jason J. Xenakis, and Lisa A. Spencer

Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115 USA

**Background:** Notch signaling is an evolutionarily conserved pathway in cell-to-cell communication. In the canonical pathway, intracellular domains released from activated Notch receptors (NICDs) translocate to the nucleus and turn on transcription of Notch-responsive genes. We previously reported mature human eosinophils express Notch receptors and ligands, and auto-crine Notch signaling is involved in GM-CSF-induced eosinophil polarization, chemokinesis and survival [Spencer 2009 Blood. 113, 3092].

**Objectives:** The purposes of the present study are to 1) determine whether Notch signaling is required for GM-CSF-primed eosinophil transmigration across cytokine-activated endothelium, and 2) elucidate mechanisms of Notch-dependent regulation of GM-CSF-primed eosinophil functions.

**Methods:** Eosinophils were primed for 18 h in medium alone, or containing GM-CSF, with Notch inhibitors GSI (g-secretase inhibitor II), DAPT (g-secretase inhibitor IV), or DMSO as a vehicle control. Anti-Notch receptor 1 neutralizing antibody (Anti-N1) and MEK inhibitor U0126 or their appropriate controls were also included in certain experiments. For eosinophil transmigration assays, human umbilical vein endothelial cells (HUVEC) were grown to confluence on transwell inserts, and overlaid with  $0.2 \times 10^6$  primed eosinophils per well. In some wells, HUVEC monolayers were activated by 100 pM IL-4/TNF- $\alpha$  prior to transmigration. Migrated cells were counted by flow cytometry and expressed as a percentage of total input cells. Eosinophil shape changes were assessed after three hours of stimulation using the forward scatter (FSC) parameter of flow cytometry. Levels of phosphorylated ERK1/2 were measured by intracellular flow cytometry, or multiplex analysis normalized to total ERK.

**Results:** Compared to non-primed eosinophils, GM-CSF-primed eosinophils demonstrated increased transmigration through non-activated and IL-4/TNF- $\alpha$  activated endothelial monolayers ( $p < 0.001$ ,  $N = 7$ ). Exposure to GSI, DAPT, or Anti-N1 significantly inhibited GM-CSF-primed eosinophil transmigration in each condition compared to vehicle or isotype controls ( $p < 0.05$ ;  $N = 7$  (GSI),  $N = 5$  (all others)). Inhibition of Notch signaling diminished GM-CSF-induced eosinophil shape changes at three hours; in contrast, GM-CSF-induced shape changes at this time point were not affected by the protein synthesis inhibitor cycloheximide. GM-CSF-induced early ERK1/2 phosphorylation (2-5 minutes) was delayed in eosinophils exposed to GSI or aN1. Moreover, MEK 1/2 inhibitor U0126 significantly inhibited eosinophil transmigration compared to vehicle control ( $p < 0.01$ ,  $N = 6$ ).

**Conclusions:** Our data show GM-CSF-primed eosinophil transendothelial migration is dependent upon Notch signaling. Inhibition of Notch signaling prevented GM-CSF-induced eosinophil shape changes independently of protein translation, suggesting a noncanonical, cytoplasmic role for activated NICD. Inhibition of Notch signaling delayed GM-CSF-induced ERK1/2 phosphorylation, and blockade of the ERK pathway inhibited eosinophil transmigration. Taken together, these data suggest Notch signaling may regulate eosinophil transendothelial migration through noncanonical cytoplasmic effects of NICD on ERK pathway activation.

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**POSTER 29****ADIPONECTIN ATTENUATES HUMAN EOSINOPHIL ADHESION AND CHEMOTAXIS**

Shigeharu Ueki, Rie Yamamoto, Yuki Moritoki, Yoshiki Kobayashi, Hajime Oyamada, Yasunori Konno, Mami Tamaki, Masamichi Itoga, Masahide Takeda, and Junichi Chihara

Affiliation: Department of Infection, Allergy, Clinical Immunology and Laboratory Medicine, Akita University Graduate School of Medicine, Akita, Japan

**Background:** Accumulating evidence has shown an association between obesity and asthma. Adiponectin, an adipocyte-derived cytokine, is known to have anti-inflammatory effects with reduced concentrations in obese subjects. Recent findings raised the intriguing possibility that adiponectin might play a role in allergic inflammation, although the mechanistic basis for their relationship remains unclear. The purpose of this study was to examine whether adiponectin might affect functions of eosinophils, which play an important role in the pathogenesis of asthma.

**Methods:** Human peripheral blood eosinophils were purified to study expression of adiponectin receptors AdipoR1 and AdipoR2 using RT-PCR and flow cytometry. The effect of adiponectin on eosinophil survival was investigated using annexin V and propidium iodide staining. Eotaxin-induced cell adhesion was investigated using ICAM-1-coated plates. A Boyden chamber and real-time horizontal migration system were used for eotaxin-directed chemotaxis assay.

**Results:** AdipoR1 and AdipoR2 were expressed in human eosinophils. Adiponectin did not affect eosinophil survival; however, eotaxin-enhanced adhesion was inhibited by pretreatment with adiponectin. Adiponectin also diminished eotaxin-directed chemotactic responses by disturbing both velocity and directionality.

**Conclusions:** These results indicate that adiponectin attenuates the eosinophil functions induced by eotaxin without affecting cell viability. Increasing circulating adiponectin might be a novel therapeutic modality for treatment of asthma, especially in obese asthmatics.

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## POSTER 30

## TRAPPING CAPACITY AND STABILITY OF EOSINOPHIL EXTRACELLULAR DNA NETS

Shigeharu Ueki,<sup>1,2</sup> Rossana C. N. Melo,<sup>1,3</sup> Peter F. Weller<sup>1</sup>

<sup>1</sup> Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. <sup>2</sup> Department of Infection, Allergy, Clinical Immunology and Laboratory Medicine, Akita University Graduate School of Medicine, Akita, Japan. <sup>3</sup> Laboratory of Cellular Biology, Department of Biology, Federal University of Juiz de Fora (UFJF), Juiz de Fora, MG, Brazil

**Background:** Human eosinophils, that can release mitochondrial DNA, can also release nuclear DNA, the latter as filamentous histone-bearing chromatin structures through rapid, cytolytic eosinophil extracellular DNA trap cell death (eosinophil ETosis (EETosis)). Although nuclear-derived, extracellularly released DNA nets from other leukocytes are known to capture a variety of pathogens, excess DNA net formation is thought to contribute to the increased viscosity of sputum and tissue damage.

**Methods:** To study the trapping properties of eosinophil nuclear-derived DNA nets, we utilized an *in vitro* system using purified blood eosinophils in which EETosis was induced by calcium ionophore, phorbol myristate, or immobilized IgG. After eosinophil cell death, culture plates were briefly shaken and subjected to flow shear stress to visualize DNA net aggregates. Trapping of fluorescence-labeled bacteria and microbeads was studied by fluorescence microscopy and luminometer. Stability of DNA nets was assessed by fluorescence microscopy.

**Results:** Filamentous DNA nets, difficult to visualize in static culture conditions even after cytolytic EETosis, formed visible aggregates with brief experimental shear flow stresses. Both *S. aureus* and *E. coli* were similarly trapped by DNA nets under flow conditions. DNA nets preferentially captured negatively charged, hydrophobic sulfate-modified microbeads rather than positively charged, relatively hydrophilic amine-modified beads. Particle trapping was inhibited in medium contained albumin, serum or detergents, but not in medium with EDTA or high salt. Eosinophil DNA net aggregates were more stable than neutrophil DNA nets and were degraded by proteases.

**Conclusions:** The generation of extracellular DNA nets by nuclear DNA liberated by eosinophils undergoing cytolytic EETosis can trap bacteria. The trapping capacities of EETosis-induced DNA nets was most likely due to both hydrostatic, charge interactions and mostly hydrophobic interactions between DNA nets and the particles and bacteria. We also demonstrate that the trapping capacities of eosinophil nuclear-derived DNA nets are functionally susceptible to proteolytic degradation.

**Grant support:** NIH R37-AI020241 and R01-AI051645 (PFW).

## POSTER 31

**LEPTIN ACTIVATES EOSINOPHIL LEUKOTRIENE C<sub>4</sub> SYNTHESIZING MACHINERY: ROLE OF PI3K AND ENDOGENOUS RANTES**

Tatiana Luna-Gomes<sup>1</sup>; Gláucia Souza de Almeida<sup>2</sup>; Marcos Gama-Almeida<sup>1</sup>; Andrea S. Calheiros<sup>2</sup>; Peter F. Weller<sup>3</sup>; Patricia T. Bozza<sup>2</sup>; Christianne Bandeira-Melo<sup>1</sup> and Clarissa M. Maya-Monteiro<sup>2</sup>.

<sup>1</sup>Laboratório de Inflamação, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil;

<sup>2</sup>Laboratório de Imunofarmacologia, IOC, FIOCRUZ, Rio de Janeiro, RJ, Brazil;

<sup>3</sup>Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

**Background:** Leptin is an adipocytokine involved not only in the control of body weight but also in the neuro-immuno-endocrine modulation. Several leukocytes involved in immune and inflammatory conditions express functional leptin receptors, including human eosinophils. Here, we investigated potential leptin effect on eliciting eosinophil LTC<sub>4</sub> synthesis.

**Methods and results:** *In vitro* direct stimulation of human blood eosinophils with leptin elicited a rapid activation of human eosinophils. Specifically, identical to RANTES stimulation, leptin induced priming for enhanced synthesis of LTC<sub>4</sub>, but not of PGE<sub>2</sub> and dose-dependent lipid body biogenesis. Mouse eosinophils were also directly activated *in vitro* by leptin stimulation. Similar to *in vitro* data, leptin administration in actively sensitized mice induced both increased lipid body assembly within infiltrating eosinophils and LTC<sub>4</sub> production. PI3K activation within eosinophils represents part of downstream signaling involved in leptin-induced lipid body-driven LTC<sub>4</sub> synthesis, since PI3K inhibitors blocked both lipid body biogenesis, LTC<sub>4</sub> synthesis and RANTES release triggered by *in vitro* leptin stimulation. *In vivo*, PI3K deficient mice in response to leptin administration displayed reduced eosinophilic inflammation with reduced *in situ* levels of RANTES. As both *in vitro* and *in vivo* leptin-driven eosinophil activation appear to be associated with RANTES secretion, we analyzed whether endogenous RANTES mediated leptin effect. Indeed, leptin-induced activation of human eosinophils was found to be mediated by an autocrine activity of endogenous CCR3-acting RANTES, since the effects of leptin were blocked by neutralizing anti-RANTES and anti-CCR3 antibodies. Similarly, *in vivo* lipid body biogenesis within infiltrating eosinophils and LTC<sub>4</sub> production of leptin-challenged mice were significantly inhibited by pre-treatment with anti-RANTES antibody.

**Conclusions:** Altogether, our findings unveiled leptin role in activating either human or mouse eosinophil lipid body-driven LTC<sub>4</sub> synthesizing machinery, a phenomenon that involves signaling through PI3K activation and RANTES mediation. Our results establish eosinophil activation by leptin as a connection between obesity and allergy disorders.

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## POSTER 32

## THE PROSTAGLANDIN D2 RECEPTORS DP AND CRTH2 COOPERATIVELY REGULATE NFAT AND SRE

Miriam Peinhaupt<sup>1</sup>, Miriam Sedej<sup>1</sup> and Àkos Heinemann<sup>1</sup>

<sup>1</sup>Institute of Experimental and Clinical Pharmacology, Medical University of Graz, 8010 Graz

**Background:** As DP and CRTH2 were recently shown to form heteromeric signaling units and alter each other's signaling capacity<sup>1</sup>, the particular cooperation between DP and CRTH2 in controlling eosinophil function may be crucial in the development of allergic diseases like bronchial asthma. This study addresses the capacity of the interlinked DP and CRTH2 signaling to regulate the induction of the serum response element (SRE) and the nuclear factor of activated T cells (NFAT) as these elements are highly implicated in the regulation of inflammatory reactions.

**Methods:** Activation of NFAT and induction of SRE by DP- and CRTH2-mediated signaling was assessed with luciferase reporter gene assays in HEK293 cells that overexpress DP and/or CRTH2 (HEK-DP, HEK-CRTH2, HEK-DP+CRTH2). Median effective concentration values were analyzed by means of nonlinear regression with Prism 4.02 software (GraphPad Software, Inc, San Diego, Calif). Primary eosinophils were isolated from peripheral blood of healthy donors and studied with regard to adhesion under flow conditions and immunofluorescence staining of F-actin and NFAT.

**Results:** Reporter gene assays indicate that DP and CRTH2 closely interact in the activation of NFAT. DP is essential for this signaling cascade while CRTH2 has a modulatory role as activation or blockade of this receptor influenced the extent of NFAT activation. In eosinophils, treatment with specific agonists for DP and/or CRTH2 induced nuclear translocation of NFAT (rep. images from 3 ind. exp.). In HEK-DP+CRTH2 the CRTH2-mediated SRE induction is dependent on the co-expression and the functionality of DP which is therefore essential for SRE induction and can additionally induce SRE independently from CRTH2. SRE regulates the remodeling of the actin cytoskeleton and thus is crucial for cellular functions like adhesion and migration. Indeed both, DP and CRTH2 contribute to cytoskeletal rearrangement (rep. images from 3 ind. exp.) whereas the selective CRTH2 antagonist Cay10471 reduces the PGD2 induced adhesion of eosinophils under flow conditions ( $P < 0.01$ ).

**Conclusions:** The obtained data demonstrate a close interaction of DP and CRTH2 in primary eosinophils as well as in the recombinant cell model for DP and CRTH2 interaction. This study reveals that powerful signaling of CRTH2 seems to rely on the functionality of DP although CRTH2 often dominates over DP. This might be worth to be considered in approaches to treat inflammatory diseases like bronchial asthma. Developing drugs that incorporate heteromeric-receptor signaling units provides the possibility to target pathologies of the immune system more selectively and to prevent potentially harmful side effects.

<sup>1</sup> Sedej, M. et al. D-type prostanoid receptor enhances the signaling of chemoattractant receptor-homologous molecule expressed on TH2 cells. *Journal of Allergy and Clinical Immunology* 129, 492-500.e499, doi:10.1016/j.jaci.2011.08.015 (2012).

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**POSTER 33****EOSINOPHIL GRANULE STABILITY AND VIABILITY ARE CRITICALLY DEPENDENT ON CYSTATIN F**

Stephen P. Matthews, Stewart Fleming\* and Colin Watts

Division of Cell Signaling &amp; Immunology, College of Life Sciences and \*College of Medicine Dentistry and Nursing, University of Dundee, Dundee DD1 5EH, UK

Cytotoxic leukocytes such as eosinophils package toxic proteins into specialized granules for eventual discharge upon activation. How eosinophils are able to orchestrate the synthesis, activation and packaging of toxic proteins without killing themselves is not known. We have identified a critical role for an unusual protease inhibitor in eosinophil granule stability. Cystatin F is made as an inactive di-sulphide linked dimer and is converted to active monomers by protease action offering a mechanism to attenuate excessive protease activity and its consequences. We find that eosinophils in cystatin F null mice have strikingly reduced granularity and are present in lower numbers relative to wild type. Mixed bone marrow chimeras showed that the requirement for cystatin F was intrinsic to the eosinophil and that re-expression of the inhibitor rescued the phenotype. Cystatin F null eosinophils cultured *in vitro* from bone marrow also had reduced granularity but this could be restored to wild-type levels by addition of chemical cysteine protease inhibitors. Electron microscopy demonstrated abnormal eosinophil morphology in the absence of cystatin F, with fewer intact granules and extensive granules with an uncondensed or partially degranulated appearance. Our data demonstrate the importance of regulated protease activity for efficient granule toxin storage and identify cystatin F as an essential mediator of this control. We suggest that cystatin F suppresses the activity of one or more cysteine proteases that are involved in granule protein activation and/or normal packaging. Our data extend the concept recently demonstrated in CD8 T cells that cytotoxic leukocytes utilize specific protease inhibitors to avoid self-inflicted toxicity.

*Supported by the Wellcome Trust*

## POSTER 34

## LECTIN AFFINITY-BASED SEPARATION OF VARIANTS OF EOSINOPHIL CATIONIC PROTEIN (ECP)

Jenny Rubin<sup>1</sup>, Per Venge<sup>1</sup> and Rodolfo C. Garcia<sup>1</sup>

<sup>1</sup>Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden.

**Background:** Eosinophil Cationic Protein (ECP) is a glycosylated protein found in the secondary granules of eosinophil granulocytes. Native ECP differs in molecular weight from approximately 16-22 kDa due to different post-translational modifications such as glycosylation; there are three sites for N-linked glycosylation on ECP.

By Surface- Enhanced Laser Desorption/Ionization- Time of Flight Mass Spectrometry (SELDI-TOF MS), we have previously identified ~10 different molecular species ranging from 15.7-17.9 kDa. We have also found that less glycosylated ECP, of lower molecular mass, is more cytotoxic *in vitro*.

The current method of purifying and fractionating native ECP by gel filtration and ion exchange chromatography does not allow a full identification of each molecular variant of ECP since they differ so little in size and charge. The present project aims at developing lectin affinity-based purification methods relying on the composition and structure of the carbohydrate chains linked to the protein backbone. This would enable further structural studies as well as establishing relationships between the carbohydrate structure of different molecular variants and their *in vitro* activity.

**Methods:** Native ECP was extracted from buffy coats of healthy blood donors and was fractionated by gel filtration and ion exchange chromatography. A pool of ECP fractions of high molecular mass were examined by SELDI-TOF MS, showing the presence of molecular variants of 16.1 to 17.4 kDa.

Five different agarose-bound lectins were selected for their potential to interact with carbohydrate moieties on the surface of ECP. The ECP pool was sequentially incubated with each of the agarose-bound lectins, starting with that corresponding to the presumed outermost carbohydrate moiety. ECP bound to the various lectins was eluted with specific sugars and the eluted fractions were assayed for ECP concentration by ELISA as well as analyzed by SELDI-TOF MS.

**Results:** Two out of the five different agarose- bound lectins bound ECP-variants with relative specificity. A sialic acid-specific lectin captured an ECP-variant of molecular mass 17.43 kDa and an N- acetylglucosamine-specific lectin captured an ECP-variant of molecular mass 17.17 kDa. Lectins with affinity for carbohydrate moieties presumed to be located closer to the protein backbone in the N-linked carbohydrate chain did not capture preferentially ECP-variants of lower molecular mass, as had been expected.

**Conclusions:** The results obtained so far indicate that lectins could indeed be a useful tool to separate molecular variants of ECP. Further testing is still needed to find lectins capable of capturing every one of the ECP-variants. Additionally, the molecular masses of the variants that were found suggest that only one of the N-linked glycosylation sites is glycosylated per ECP-molecule.

**Grant support:** *This study is supported by grants from the Swedish Medical Research Council and the Swedish Heart and Lung Foundation.*

**POSTER 35****INVOLVEMENT OF THE CANNABINOID RECEPTOR 2 IN THE ACTIVATION AND CHEMOTAXIS OF HUMAN EOSINOPHILS**

Robert Frej, Eva M. Sturm, Àkos Heinemann

**Background:** Evidence has accumulated that cannabinoids, especially CB2-receptor ligands may play a major role in mediating inflammatory responses. Human peripheral blood eosinophils express only CB2 receptors, but their role in inflammation is still poorly defined.

**Methods:** Blood was sampled from healthy volunteers, erythrocytes were removed by dextran sedimentation and polymorphonuclear leukocytes were obtained via Histopaque gradients. For all assays eosinophils were further purified by negative magnetic isolation. Shape change was recorded immediately after 4 min stimulation on a FACSCalibur flow cytometer. Up-regulation and activation of integrin  $\alpha M\beta 2$  (Mac-1) was also recorded by flow cytometry. An AP48 microBoyden chamber was used for chemotaxis assays and migration time was 1h at 37°C.

**Results:** To assess eosinophil activation, shape change assays were done and showed significant enhancement ( $p < 0.001$ ) by pre-treatment with the selective CB2-receptor agonist JWH-133 at nanomolar concentrations. As shape change indicates cytoskeletal rearrangement, which is required for firm arrest and subsequent transmigration of eosinophils, chemotaxis assays were conducted and showed that JWH-133 significantly ( $p < 0.01$ ) enhanced migration towards prostaglandin (PG) D2 and eotaxin-2. Furthermore the up-regulation and activation of integrin  $\alpha M\beta 2$  (Mac-1) by PGD2 or eotaxin-2 was increased by pre-treating eosinophils with JWH-133 ( $p < 0.01$ ).

**Conclusion:** Our results suggest that activating CB2 plays a role in the activation of human peripheral blood eosinophils. A hallmark of allergic inflammatory diseases like asthma and atopic diseases is the accumulation of eosinophils in the tissue, to which the endocannabinoid system may contribute to in a still unknown extent via modulation of eosinophil activation and chemotaxis.

*Acknowledgments: This study was supported by the Jubiläumsfonds of the Austrian National Bank (OeNB, grants 14446 and 14263) and the Austrian Science Fund (FWF, grant P22521).*

## POSTER 36

**THE EFFECT OF HMGB1 ON MOUSE EOSINOPHILS: VIABILITY, CHEMOTAXIS AND THE EXPRESSION OF RAGE, TLR2 AND TLR4**

Kimberly D. Dyer and Helene F. Rosenberg

Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892 USA

**Background:** Lotfi *et al.* (1) demonstrated that human eosinophils are mobilized and activated in response to the alarmin HMGB1 (high mobility group box 1) and proposed a role for endogenous DAMPs in the induction of eosinophilic inflammation. We are interested in exploring the possibility that HMGB1 may play an important role in non-IL5 dependent eosinophilias, potentially those associated with myalgias and myopathies (2). Toward the goal of exploring this issue in a physiologic setting, we examine the biology of HMGB1 and its characterized receptors, RAGE (receptor for advanced glycation end-products), as well as TLR2 and TLR4 in mouse eosinophils.

**Methods:** Eosinophils were generated from the bone marrow of wild-type BALB/c mice (3) or isolated from the spleen of the IL-5 transgenic mice (4) and subjected to flow cytometric analysis for the expression of RAGE, TLR2 and TLR4 in conjunction with the expression of Siglec F. Mouse eosinophils were evaluated in a chemotaxis assay with recombinant HMGB1 (0.01 – 10 mg/ml) or eotaxin (0.01 – 10 mg/ml) as a positive control for 2 hours and migration through a 5 µm filter was assessed. Mouse eosinophils were incubated with HMGB1 (0.01 – 10 mg/ml) or IL-5 (0.01 – 10 mg/ml) as a positive control for 24 or 48 hours and viability was assessed.

**Results:** HMGB1 (10 µg/ml) failed to maintain the viability of the BALB/c mouse eosinophils as compared to the negative (no cytokine support) control. HMGB1 elicited minimal chemotaxis of the BALB/c mouse eosinophils at the highest concentration tested (10 µg/ml). Very few eosinophils from BALB/c mice express RAGE and TLR4 and no TLR2 expression was detected in the Siglec F-positive eosinophil population.

**Conclusions:** Only two of the known receptors for HMGB1- RAGE and TLR4 – are expressed, and only on a very small population BALB/c mouse eosinophils from the two sources examined. We will explore the possibility that HMGB1 responses and receptor expression may be mouse strain dependent and/or may require eosinophil priming or activation *in vivo*.

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## POSTER 37

**THE C-TERMINUS OF CCR3 MODULATES CELL SURFACE EXPRESSION AND FUNCTIONAL RESPONSES TO THE EOTAXIN FAMILY OF CHEMOKINES**

Pallavi Patel<sup>1,2</sup>, Roberto Solari<sup>2,3</sup>, Tomoko Tsuchiya<sup>4</sup>, Shiro Kanegasaki<sup>4</sup> and James E. Pease<sup>1,2</sup>

<sup>1</sup>Leukocyte Biology Section, National Heart and Lung Institute, Faculty of Medicine, Imperial College London, South Kensington Campus, London, SW7 2AZ, UK. <sup>2</sup>Medical Research Council/Asthma UK Centre in Allergic Mechanisms of Asthma, UK. <sup>3</sup>Infection and Airways Disease Group, Respiratory Infections Section, National Heart and Lung Institute, Faculty of Medicine, Imperial College London, St Mary's Campus, Norfolk place, London, W2 1PG, UK. <sup>4</sup>YU-ECI Research Center for Medical Science, Yeungnam University, Gyeongsansi, 712-0096, Republic of Korea.

**Background:** The chemokine receptor CCR3 is a key mediator of eosinophil migration, transducing responses to the eotaxin family of chemokines. Previously, we have reported that a single nucleotide polymorphism (SNP) T971C, encoding a point mutation in the CCR3 C-terminus, severely impairs receptor trafficking to the cell surface. We describe here the results of further studies to define the role of the C-terminus of CCR3 in the regulation of receptor expression and function.

**Methods:** CCR3 function on eosinophils was examined by shape change assay and realtime chemotaxis measurements using TAXIScan apparatus. Chimeric constructs in which the C-terminus of CCR3 and the related receptor CCR1 were exchanged were generated by PCR and transiently expressed in a murine pre-B cell line. A mutant CCR3 containing the naturally occurring SNP T971C was also examined. Cells were subsequently assessed for cell-surface expression and chemotaxis via Boyden chambers.

**Results:** The three eotaxins, CCL1, CCL24 and CCL26 displayed significantly different potencies and efficacies in assays of chemotaxis and eosinophil shape change. Replacement of the CCR1 C-terminus with that of CCR3 lead to a significant reduction in expression. The reciprocal chimera also trended towards lower expression. Both constructs were still able to mediate chemotaxis to their cognate ligands. The misfolded CCR3 mutant encoded by the T971C mutant was predominantly retained intracellularly, but cell surface expression could be rescued in a dose dependent manner by incubating cells with the small molecule CCR3 antagonist UCB 35625.

**Conclusions:** We hypothesize that the three structurally diverse eotaxins stabilize CCR3 in distinct conformations, leading to distinct signaling outcomes and the observed differences in potency and efficacy. The C-terminal region of CCR3 dictates the expression and function of CCR3, most likely by coupling to specific chaperones and intracellular signaling machinery such as G proteins. Small molecule antagonists of CCR3 have additional therapeutic function by acting as pharmacoperones, providing a structure around which mutant CCR3 can be properly folded.

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## POSTER 38

## CALCITRIOL REDUCES EOSINOPHIL CYTOLYSIS AND RELEASE OF CYTOTOXIC GRANULES

Caroline Ethier<sup>1</sup>, Paige Lacy<sup>1</sup>, Francis Davoine<sup>1,2</sup>.

Department of Medicine<sup>1</sup>, Campus Saint-Jean<sup>2</sup>, University of Alberta, Edmonton, Alberta, Canada.

**Background:** Epidemiological studies correlate vitamin D deficiency with asthma severity. Direct evidence supporting a role for vitamin D in asthma has also been revealed from genetic studies of vitamin D receptor polymorphisms as a risk factor for asthma in different human populations. Calcitriol (1,25-dihydroxyvitamin D3) has many physiological roles, and has recently been shown to modulate receptor and cytokine expression in leukocytes. Despite the association of vitamin D isoforms with allergic asthma, little is known about the direct effects of calcitriol on eosinophils. We hypothesized that calcitriol has direct effects on eosinophil survival and effector responses.

**Objectives:** To determine the effects of the physiologically active form of vitamin D, calcitriol, on human eosinophil survival and degranulation responses.

**Methods:** Peripheral blood eosinophils from asthmatic atopic donors were isolated and incubated with calcitriol and anti-apoptotic factors (interleukin-5 (IL-5) or interferon-g (IFNg)). Dose-response assays were completed using physiological doses of calcitriol (0.01-100 nM). The additive effects of calcitriol and IFNg (100 ng/mL) or IL-5 (1ng/mL) were investigated using 7- and 14-day incubations, respectively. Viability/apoptosis/ necrosis levels were measured using an Annexin-V/PI assay. Degranulation was measured by cytolysis, determined by the presence of free granules was observed by flow cytometry using EPX monoclonal antibody, or by EPX activity using a colorimetric assay with OPD substrate.

**Results:** Treatment of human eosinophils with calcitriol alone yielded similarly reduced viability ( $4.5 \pm 1.5\%$ ,  $n = 12$ ) after 7 days compared to control media ( $2.1 \pm 2.1\%$ ,  $n = 12$ ). In contrast, calcitriol was able to increase viability in eosinophils incubated with IL-5 in a synergistic manner for up to two weeks. After 14 days of incubation,  $66 \pm 7\%$  of eosinophils were still intact when treated with calcitriol (10 nM) and IL-5 (1 ng/ml), compared to IL-5 alone ( $34 \pm 8\%$ ,  $p < 0.05$ ,  $n = 4$ ). Cells treated with IL-5 alone showed a greater increase in necrosis after 7 days compared with those treated with IL-5/calcitriol by up to 32% at day 14 of incubation. A similar trend ( $p = 0.091$ ) of reduction of necrosis was observed when calcitriol was added to IFNg. Increased eosinophil survival in the presence of calcitriol and IL-5 strongly correlated with reduced necrosis and cytolytic release of EPX-positive granules which appeared in cell culture media as determined by flow cytometry. Finally, we measured EPX activity in culture media after 4 and 7 days and following platelet activating factor (PAF)-induced degranulation. Calcitriol co-incubated with IL-5 reduced EPX activity in media after 7 days (3.4-fold decrease), compared with IL-5-treated eosinophils ( $n = 3$ ). Similarly, a 1.6-fold reduction in EPX activity was observed in PAF-induced degranulation in calcitriol/IL-5 treated eosinophils.

**Conclusions:** Our findings suggest an anti-inflammatory role for calcitriol, which prevents eosinophil cytolysis and release of granules while promoting eosinophil survival or apoptosis. Sustaining eosinophil viability while reducing necrosis in favour of apoptosis may decrease cytotoxic granule release into tissues and mucosa in allergic inflammation, therefore reducing mucosal inflammation.

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## POSTER 39

**N-GLYCOSYLATION OF NOX2 MEDIATES ITS TRAFFICKING TO THE CELL SURFACE AND INDUCES ROS-DEPENDENT SURFACE UP-REGULATION OF BLT1 DURING EXOCYTOTIC DEGRANULATION IN HUMAN EOSINOPHILS STIMULATED WITH LTB<sub>4</sub>**

Arim Min, Myeong Heon Shin

Department of Environmental Medical Biology, Institute of Tropical Medicine, and Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea, 120-752

Exocytosis-mediated degranulation in human eosinophils contributes to allergic inflammation. LTB<sub>4</sub> is a pro-inflammatory lipid mediator to elicit eosinophil exocytosis. However, detailed signaling mechanisms of eosinophil exocytosis induced by LTB<sub>4</sub> are poorly understood. Herein, we report that N-glycosylation-dependent surface trafficking of NOX2 play an important role in ROS-dependent surface upregulation of high affinity LTB<sub>4</sub> receptor BLT1 leading to exocytosis in human eosinophils induced by LTB<sub>4</sub>. Stimulation of eosinophils with LTB<sub>4</sub> induced production of intracellular ROS, surface upregulation of exocytosis marker protein CD63 via BLT1. LTB<sub>4</sub> induced phosphorylation of p47<sup>phox</sup>, and inhibition of ROS generation with NOX2 inhibitors prevented LTB<sub>4</sub>-stimulated exocytosis. LTB<sub>4</sub> increased expression of BLT1 and NOX2 at the cell surface, which peaked at 30 min after stimulation. LTB<sub>4</sub>-triggered surface upregulation of BLT1 and NOX2 was blocked by pretreatment with NOX2 inhibitors and BLT1 antagonist, respectively. Interestingly, glycosylated NOX2 at 91 kDa was highly expressed at 30 min after LTB<sub>4</sub> stimulation. Inhibition of LTB<sub>4</sub>-induced NOX2 glycosylation with N-glycosylation inhibitor, tunicamycin led to disability of NOX2 to travel to the cell surface, which resulted in marked reduction of LTB<sub>4</sub>-induced ROS generation, surface up-regulation of BLT1 and exocytosis. Finally, inhibition of LTB<sub>4</sub>-induced exocytosis with PKC inhibitor, Src kinase inhibitor or lipid rafts inhibitor suppressed surface trafficking of NOX2, ROS generation, and surface upregulation of BLT1. These results suggest that BLT1-mediated N-glycosylation of NOX2 play an important role in its surface trafficking, which can regulate ROS-dependent exocytosis in LTB<sub>4</sub>-stimulated eosinophils.

## POSTER 40

**CHARACTERIZATION OF THE INFLAMMASOME PROTEINS IN HUMAN EOSINOPHILS**

Renata T. Nesi,<sup>1,2</sup> Revital Shamri,<sup>1</sup> Kristen Young,<sup>1</sup> Samuel S. Valença<sup>2</sup>, Josiane S Neves,<sup>2</sup> Peter F. Weller<sup>1</sup>

<sup>1</sup>Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA; <sup>2</sup>Institute of Biomedical Sciences, Federal University of Rio de Janeiro, RJ, Brazil.

**Background:** Inflammasomes are caspase-1-activating multiprotein platforms that control maturation and secretion of interleukins, such as IL-1 $\beta$  and IL-18, to induce proinflammatory activities as part of host responses to infection and injury. Most studies to date have characterized inflammasomes in innate immune cells of the myeloid lineage, such as macrophages or dendritic cells. However, other cells outside the myeloid compartment can activate inflammasomes. Activation of inflammasome proteins is induced through the pattern-recognition receptors, such as the members of the NOD-like receptor family (NLR). Eosinophils express and secrete IL-1 $\beta$ , moreover, human eosinophils express Nod 1 and Nod 2 receptors, but not NLRP3, but only a few studies have investigated the inflammasome platforms in eosinophils. Therefore, our studies aimed to better characterize the inflammasome machinery in eosinophils, and its role in eosinophil-mediated host defense, immunoregulation or pathology.

**Methods:** Western blotting, RT-PCR and ELISA methods were utilized to determine the expression and function of inflammasome proteins in human eosinophils isolated from peripheral blood of healthy donors.

**Results:** Human eosinophils contained IL-18 as a preformed cytokine, and both the pro and cleaved forms of IL-18 were found in human eosinophils. Preliminary stimulation studies showed varied results regarding the ability of GM-CSF to increase expression and secretion of IL-18 from human eosinophils. Additionally, the expression of the inflammasome proteins, NLRP10 and NLRP12, was not found in purified human eosinophils, suggesting human eosinophils utilize other members of the NLR family to activate their inflammasome machinery, or require an additional signal to induce their expression.

**Conclusions:** Our results showed that human peripheral blood eosinophils express the pro and cleaved forms of IL-18, ready to be secreted. This preformed IL-18 expression is not accompanied with expression of NLRP10 and NLRP12 receptors.

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## POSTER 41

**THE ROLE OF CRYPTOCOCCUS NEOFORMANS CAPSULE IN MODULATION OF EOSINOPHILS ACTIVATION**

Thaís Amanda de Pinho Silva<sup>1</sup>, Raquel das Neves Almeida<sup>1</sup>, Tatiana Luna<sup>2</sup>, Célio Geraldo Freire de Lima<sup>4</sup>, Patricia Torres Bozza<sup>3</sup>, Christianne Bandeira-Melo<sup>2</sup> and Kelly G. Magalhães<sup>1</sup>

<sup>1</sup>Lab. de Imunologia e Inflamação, Universidade de Brasília, UnB, Brazil; <sup>2</sup> Lab. Inflamação, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brazil; <sup>3</sup>Lab. Imunofarmacologia, Instituto Oswaldo Cruz, FIOCRUZ, Brazil; <sup>4</sup>Lab. de Biologia Imunitária, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brazil.

**Background:** *Cryptococcus neoformans* is a human pathogenic yeast, which causes pneumonia and granulomas in immunocompromised individuals. It has already been described that some symptomatic individuals have high levels of eosinophils in systemic circulation. Furthermore, *C. neoformans* phagocytosis by eosinophils followed by cytokine release has been demonstrated in mouse infected with *C. neoformans*. However, the modulation of eosinophils lipid metabolism by *C. neoformans* and the role of this fungus capsule components in this process are not still understood.

**Objectives:** Here we investigate the role of *C. neoformans* capsule in the activation of eosinophils, investigating the modulation of lipid body biogenesis and the role of DP1, DP2 and PGD synthase in this process.

**Methods:** Purified human eosinophils or Balb/c bone marrow derived eosinophils were incubated for 2 h at 37°C with *C. neoformans* strain B3501 and its acapsular mutant Cap67. In addition, eosinophils were stimulated with *C. neoformans* capsule components GXM and GalXM and *C. neoformans*-derived lipid extract. In order to characterize the signaling pathway involved in eosinophil activation, cells were pre-treated with inhibitors of PGD synthase (HQL-79), DP1 (BW A868C), and DP2 (Cay 10471). Biogenesis of lipid bodies was determined by flow cytometer and confocal microscopy analysis, and quantification of cytokines was assessed by ELISA.

**Results:** The acapsular mutant of *C. neoformans*, but not the wild type, induced significant lipid body formation in both human and murine eosinophil. Pre-treatments of eosinophils prior to *C. neoformans* infection demonstrated that lipid body formation was dependent of DP1 and DP2 receptors as well as PGD synthase. In addition, the acapsular mutant of *C. neoformans*, but not the wild type, also induced high levels of IL-1 $\beta$  secretion in a DP2 dependent manner. Capsule components and *C. neoformans*-derived lipid extract failed to induce lipid body formation.

**Conclusions:** Our results showed that *C. neoformans* are capable of direct activation of human and murine eosinophils, triggering lipid body biogenesis and release of proinflammatory cytokines. Taken together, our findings suggest that *C. neoformans* capsule and its major components GXM and Galxm may play an important role in host immune response evasion by this fungus.

**Grant support:** CNPq

## POSTER 42

## CD48 PARTIALLY MEDIATES STAPHYLOCOCCUS AUREUS INDUCED EOSINOPHIL ACTIVATION

Yael Minai-Fleminger<sup>1</sup>, Roopesh Singh<sup>1</sup>, Helena Migelovitch<sup>1</sup>, Bernhard Homey<sup>2</sup>, Nurit Hollander<sup>3</sup>, Allon E. Moses<sup>4</sup>, Francesca Levi-Schaffer<sup>1</sup>

<sup>1</sup> Department of Pharmacology & Experimental Therapeutics, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel.

<sup>2</sup> Hautklinik, Universitätsklinikum Düsseldorf, Germany.

<sup>3</sup> Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

<sup>4</sup> Department of Clinical Microbiology and Infectious Diseases, Hadassah University Hospital, Jerusalem, Israel.

**Background:** Allergic diseases are characterized by eosinophil tissue infiltration which is sometimes mediated by bacterial infection. Atopic dermatitis (AD) is typically associated with infection by *Staphylococcus aureus* (*S. aureus*). The mechanisms by which *S. aureus* and/or its toxins specifically interact with eosinophils and hence might exacerbate the allergic inflammation are not fully characterized as yet. Our hypothesis was that direct activating interactions between *S. aureus*/toxins and eosinophils are important and are mediated by CD48. CD48 is a glycosylphosphatidylinositol-anchored receptor belonging to the CD2 family, which we previously found to be increased in allergic diseases especially as expressed by the eosinophils. Moreover, as displayed by mast cells, CD48 was described to be important for their stimulating cross-talk with *S. aureus*.

**Methods:** In this work we investigated the specific role of CD48 expressed on human and mouse eosinophils in their interaction with *S. aureus* (heat killed) and its toxins (SEB/protein A/PGN). These interactions were analyzed *in vitro* by confocal microscopy, adhesion and degranulation assay, cell viability, cytokine release and cell signaling. In addition, *in vivo* model of peritonitis was induced by SEB to evaluate CD48 role in disease severity.

**Results:** Our results provide evidence for the recognition and direct physical interaction between eosinophils and *S. aureus*/toxins. Specific staining for CD48 and eosinophils in the skin of AD patients revealed a striking increase of CD48 eosinophil associated expression. By *in vitro* analysis, *S. aureus*/toxins were found to bind to human peripheral blood eosinophils, increase the CD48 receptor expression, and cause the release of granular mediators such as EPO, ECP,  $\beta$ -Hex, and of both pro-inflammatory, i.e. IL-8, and anti-inflammatory, i.e. IL-10, cytokines by the eosinophils. Blocking CD48 on human peripheral blood eosinophils or by analyzing bone marrow eosinophils from CD48 knock out (KO) mice revealed that these effects were significantly decreased. Moreover, CD48KO BMEos bound significantly less *S. aureus* when co-cultured *in vitro* and when incubated with the toxins released significantly less EPO. Furthermore, using a mouse model of SEB induced peritonitis in CD48 KO, we have shown the mediating role of CD48 on SEB induced total peritoneal cell and eosinophil infiltration and activation.

**Conclusions:** Our data would demonstrate the important role of CD48 in *S. aureus*/toxins-eosinophils activating interactions that can take place in allergic diseases. Therefore we view CD48 as a novel therapeutic target in the treatment of allergic diseases and especially of AD in which tissue eosinophilia and infection with *S. aureus* are present.

## POSTER 43

**AN ESSENTIAL ROLE FOR RAB27A IN EOSINOPHIL EXOCYTOSIS: IMPLICATIONS FOR AIRWAY HYPERRESPONSIVENESS**

John Dongil Kim<sup>1</sup>, Lian Willetts<sup>1</sup>, Sergei Ochkur<sup>4</sup>, Miguel C. Seabra<sup>3</sup>, Redwan Moqbel<sup>2\*</sup>, James J. Lee<sup>4\*</sup>, and Paige Lacy<sup>1\*</sup>

<sup>1</sup>Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB, Canada, <sup>2</sup>Department of Immunology, University of Manitoba, Winnipeg, MB, Canada, <sup>3</sup>Clinical Neuroscience, Division of Neuroscience and Mental Health, National Heart and Lung Institute, Imperial College London, London, UK, and <sup>4</sup>Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, AZ, USA. \* Co-supervisors of this project.

**Background:** Eosinophil degranulation is proposed to be a critical mechanism in allergic airway diseases by contributing to airway hyperresponsiveness, edema, and tissue inflammation. The Rab-related guanosine triphosphatase (GTPase) Rab27a regulates secretory vesicle trafficking in leukocytes. Our findings in human eosinophils indicate that Rab27a mRNA and protein, and that this is activated by binding to GTP upon stimulation of eosinophils with sIgA-coated beads. Here, we hypothesize that Rab27a is essential for regulating exocytosis of eosinophil crystalloid granules.

**Methods:** Eosinophils were isolated from IL-5 transgenic (IL-5/WT) and IL-5 transgenic crossed with Rab27a-deficient mice (IL-5/*Ashen*) and stimulated with platelet-activating factor (PAF) ± ionomycin to determine their ability to release eosinophil peroxidase (EPX) *in vitro*. *Ex vivo* and *in vivo* EPX release from IL-5/WT and IL-5/*Ashen* eosinophils was also determined utilizing IL-5/hE2/EPX<sup>-/-</sup> transgenic mice, which better represent human asthma patients and their characteristics such as eosinophil degranulation. The *ex vivo* study was performed by co-incubating IL-5/WT and IL-5/*Ashen* eosinophils with bronchoalveolar lavage (BAL) fluid from IL-5/hE2/EPX<sup>-/-</sup> mice. In addition, IL-5/*Ashen* eosinophils were intratracheally instilled into the lungs of IL-5/hE2/EPX<sup>-/-</sup> mice to analyze *in vivo* responses. EPX levels were measured in bronchoalveolar lavage (BAL) fluid using a novel and validated EPX ELISA developed in our lab. Finally, we compared methacholine-induced airway hyperresponsiveness in an established allergen challenge model using ovalbumin (OVA) in WT and *Ashen* mice.

**Results:** We confirmed that Rab27a was localized to eosinophil crystalloid granules by subcellular fractionation. Eosinophils lacking Rab27a expression (from IL-5/*Ashen* mice) showed significantly reduced EPX release compared with wild type eosinophils following stimulation with PAF and ionomycin ( $n = 5-10$ ,  $p < 0.001$ ). Our *In vivo* and *ex vivo* studies also showed that IL-5/*Ashen* eosinophils released less EPX than wild type eosinophils ( $n = 4$ ,  $p < 0.001$ ). Finally, in OVA-sensitized WT and *Ashen* mice, saline-challenged WT and *Ashen* exhibited similar responses, whereas OVA-treated *Ashen* showed reduced airway hyperresponsiveness than OVA-treated WT ( $n = 6-10$ ,  $p < 0.05$ ).

**Conclusions:** These findings provide important evidence that Rab27a regulates crystalloid granule trafficking and exocytosis in eosinophils. Gene deletion of Rab27a resulted in partial loss of degranulation from eosinophils, suggesting the existence of compensatory signaling mechanisms that maintain granule trafficking. Partial inhibition of degranulation by Rab27a from eosinophils was associated with reduced airway hyperresponsiveness in an allergen sensitization and challenge model in mice. These findings suggest that eosinophil Rab27a may be an important regulator of eosinophil-related allergy and asthma.

**Grant support:** This project was funded by grants from the Canadian Institutes of Health Research, The Lung Association of Alberta and NWT, and Mayo Foundation for Medical Education and Research.

## POSTER 44

**2-ARACHIDONOYL-GLYCEROL ACTIVATES HUMAN EOSINOPHILS: INVOLVEMENT OF IL-5, CB<sub>2</sub> RECEPTORS, AND EICOSANOIDS**

Michel Lavolette, Caroline Turcotte, Marie-Chantal Larose, Claudine Ferland, Cyril Martin, Véronique Provost, Nicolas Flamand.

Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Université Laval, Québec, Canada.

**Background:** The two main endocannabinoids, 2-arachidonoyl-glycerol (2-AG) and N-arachidonyl-ethanolamine (AEA), modulate inflammatory cell functions. This is linked to CB receptor activation and production of eicosanoids such as prostaglandins, eoxins, and leukotrienes, through the release of the arachidonic acid molecule present in their structure. Given eosinophils express CB<sub>2</sub> receptors, endocannabinoid hydrolyzing enzymes and eicosanoid biosynthetic enzymes, we postulated endocannabinoids activate them through multiple signalling pathways.

**Aim of the study:** To define the impact of endocannabinoids on eosinophil functions and the involved cellular mechanisms.

**Results:** 2-AG but not AEA rapidly induced the migration of eosinophils to a similar extent as CCL11. This required the presence of IL-5 and Src kinases, in contrast to 5-oxo-eicosatetraenoate (5-oxo-EET). Although eosinophils express CB<sub>2</sub> receptors, their blockade only diminished the effect of 2-AG by ~50%. CB<sub>2</sub> receptor agonists did not induce eosinophil migration but their combination with arachidonic acid led to similar migration rates to those observed with 2-AG. In this respect, 2-AG hydrolysis inhibitors that decreased arachidonic acid availability prevented the effects of 2-AG. Moreover, 2-AG induced the biosynthesis of eicosanoids (LTC<sub>4</sub> and eoxin C<sub>4</sub>). The blockade of eicosanoid biosynthetic pathways indicated that a 15-lipoxygenase metabolite might be involved in the effects of 2-AG on eosinophils. However, the 15-lipoxygenase metabolites tested so far could not mimic the effects of 2-AG.

**Conclusions:** Altogether, our data indicate that 2-AG activates human eosinophils *ex vivo*. This activation requires IL-5, implicates its hydrolysis into arachidonic acid, the activation of the 15-LO pathway and the activation of cannabinoid receptors (migration). The data support a stimulatory role of 2-AG in the regulation of eosinophil functions and might be involved in eosinophil homing to the tissue.

**Acknowledgements:** *This work was supported by grants from the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research.*

## POSTER 45

**CMRF-35-LIKE MOLECULE 1 (CLM-1) REGULATES EOSINOPHIL HOMEOSTASIS BY SUPPRESSING CELLULAR CHEMOTAXIS**

Moshkovits Itay<sup>1</sup>, Shik Dana<sup>1</sup>, Itan Michal<sup>1</sup>, Alon Y. Hershko<sup>2</sup>, van Lookeren Campagne Menno<sup>3</sup>, Munitz Ariel<sup>1</sup>

<sup>1</sup> Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, The Tel-Aviv University, Ramat Aviv, Israel.

<sup>2</sup> Laboratory of Allergy and Clinical Immunology, Department of Medicine, The Herbert Center of Mast Cell Disorders, Meir Medical Center, Kfar Saba, Israel.

<sup>3</sup> Department of Immunology, Genentech Inc., South San Francisco, CA.

**Background:** Eosinophil accumulation in health and disease is a hallmark characteristic of mucosal immunity and Th2 inflammation. Although eotaxin-induced CCR3 signaling is critical in eosinophil homing, the role of tyrosine-based inhibitory-motif-containing receptors such as CMRF-like-molecule-1 (CLM-1) that may suppress eotaxin-induced responses in eosinophils are largely unknown. We aim to define the expression and function of CLM-1 in eosinophils.

**Methods:** CLM-1 and CLM-8 expression was assessed in murine and human eosinophils (flow cytometry). The chemotactic responses of wild-type and *Clm1*<sup>-/-</sup> cells were examined *in vitro* (using trans-wells) and *in vivo* (using direct chemokine administration). CLM-1-ligand interactions were neutralized in-vitro and during allergic airway disease and the accumulation of airway eosinophils was determined.

**Results:** CLM-1 (but not CLM-8) was highly and distinctly expressed by tissue eosinophils. Strikingly, *Clm1*<sup>-/-</sup> mice displayed elevated baseline tissue eosinophilia. CLM-1 negatively regulated eotaxin induced eosinophil responses resulting in increased eosinophil chemotaxis, actin polymerization, calcium influx and ERK 1/2 but not p38 phosphorylation. Blockade of CLM-1-ligand interactions was capable of rendering wild type eosinophils hyper-chemotactic in-vitro and in-vivo during the effector phase of allergic airway disease. Suppression of cellular recruitment was specific to eosinophils and eotaxin since LTB4- and MIP-1 $\alpha$ -induced eosinophil and neutrophil migration were not regulated by CLM-1. Peripheral blood eosinophils obtained from allergic rhinitis patients displayed elevated CLM-1/CD300f levels.

**Conclusions:** CLM-1 is a novel regulator of eosinophil homeostasis by suppressing their cellular response to eotaxin. These data may ultimately lead to receptor-based tools for future therapeutic intervention in eosinophil-associated diseases.



## POSTER 46

## CMRF35-LIKE MOLECULE 1 (CLM-1) IS REQUIRED FOR IL-33-INDUCED EOSINOPHIL ACTIVATION

Dana Shik<sup>1</sup>, Ariel Munitz<sup>1</sup><sup>1</sup>Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Israel.

**Background:** Eosinophils are pleiotropic cells that accumulate in various inflammatory settings including parasitic infections, allergic diseases, colitis and cancer. In these settings a complex network of activating and inhibitory cell surface receptors “fine tune” eosinophil responses. A common phenomenon of eosinophil accumulation is their association with sites of tissue damage and dying cells, which can elicit eosinophil activation and subsequent mediator release. The alarmin cytokine IL-33 is released upon epithelial cell damage or cell death and can potently activate eosinophil mediator release, survival and signaling. CMRF35-like molecule (CLM)-1 and CLM-8 are immunoreceptor tyrosine-based inhibitory motif (ITIM)-bearing receptors that are expressed and functional in eosinophils. Recent studies have demonstrated that CLM-1 and CLM-8 can bind phosphatidylserine, a well-described marker for dying cells. Thus, we hypothesized that CLM-1 and/or CLM-8 would regulate the functional activities of eosinophils in settings of tissue damage and in particular in response to IL-33.

**Methods:** CLM-1 and CLM-8 were biochemically characterized in lysed eosinophils generated from wild type bone marrow (BM) by western blot. CLM-1 and CLM-8 expression was assessed by a flow cytometric approach in murine BM-eosinophils and mast cells (MCs) as well as in resident peritoneal eosinophils at baseline and following diverse stimuli. Wild type, *Clm1*<sup>-/-</sup> and *Clm8*<sup>-/-</sup> eosinophils and MCs were activated with IL-33 (0-100 ng/ml) and subsequent mediator release was assessed (ELISA). IL-33-induced signaling was assessed using phosphoflow. The expression of CLM-1, ST2 and IL-1RAP was examined in wild type, *Clm1*<sup>-/-</sup> and *Clm8*<sup>-/-</sup> BM-derived eosinophils (qPCR and flow cytometry).

**Results:** Biochemical analysis of CLM-1 and CLM-8 in BM-derived eosinophils demonstrated that the molecular weight of CLM-1 is 60kDa and of CLM-8 is 60 and 33kDa in eosinophils. Interestingly, CLM-8 but not CLM-1 was glycosylated as demonstrated by treatment with N-glycosidase. IL-33 (but not IL-1b, IL-25, IL-4, IL-13 and TNFa) increased the expression of CLM-1 and CLM-8 on the surface of BM-derived and resident peritoneal eosinophils in a time and concentration-dependent fashion. Assessment of IL-33-induced mediator release in wild type, *Clm1*<sup>-/-</sup> and *Clm8*<sup>-/-</sup> BM-derived eosinophils revealed that unexpectedly, CLM-1 (and to a lesser extent CLM-8) was required for IL-33-induced IL-6, IL-13, IL-4, CCL17 and Relm-a secretion. Consistently, IL-33-induced phosphorylation of NFkB and p38 was dramatically decreased in *Clm1*<sup>-/-</sup> and *Clm8*<sup>-/-</sup> BM-derived eosinophils. Decreased responsiveness to IL-33 was not due to lack of IL-33-associated signaling molecules as the expression of ST2 and IL-1RAP was similar in wild type, *Clm1*<sup>-/-</sup> and *Clm8*<sup>-/-</sup> mice. Consistently, *Clm1*<sup>-/-</sup> and to a lesser extent *Clm8*<sup>-/-</sup> BM-derived MCs displayed impaired IL-33-induced mediator release.

**Conclusions:** Our data demonstrate that CLM-1 is a novel regulator that is required for IL-33-induced cellular responses in eosinophils and mast cells. These findings suggest that CLM-1 is required for IL-33-induced eosinophil activation in settings of tissue damage and therefore may have profound implications for the design of future therapeutic tools in eosinophil and IL-33 associated diseases.

## POSTER 47

**EOSINOPHIL PHENOTYPE IN ULCERATIVE COLITIS WITH CONCOMITANT PRIMARY SCLEROSING CHOLANGITIS DEPENDS ON T-CELL SUBSETS IN THE INTESTINE**

Maria Lampinen, Annika Fredricsson, Fredrik Rorsman, Marie Carlson

Department of Medical Sciences, Gastroenterology Research Group, University Hospital, S-751 85 Uppsala, Sweden.

**Background and aim:** Primary sclerosing cholangitis (PSC) is a chronic bile duct disease characterized by obliterative fibrosis, eventually leading to cirrhosis and with increased risk of colon cancer and colangiocarcinoma. There is currently no effective medical treatment; liver transplant is the only curative option. Approximately 75% of patients with PSC have IBD, primarily ulcerative colitis (UC); about 5% of the IBD patients have concomitant PSC. However, the reason for this link is unclear. The aim of this study is to characterize the intestinal inflammation in IBD-PSC as compared to IBD without PSC. This is an ongoing study, and we present here some preliminary data on eosinophil granulocytes.

**Materials and methods:** So far we have included 8 patients with IBD-PSC (whereof 5 with active intestinal inflammation), 10 patients with UC (3 active) and 7 controls. Biopsy samples from terminal ileum and rectum were collected. Cell suspensions were prepared and analysed by flow cytometry. Eosinophils were identified as CD15<sup>+</sup>CCR3<sup>+</sup> cells, and the expression of activation markers CD44, CD69 and CD66b were analyzed. In the same samples, CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were enumerated, and the expression of Th2-profile marker CRTH2 and the Th1-profile marker CXCR3 were evaluated on CD4<sup>+</sup> T-cells.

**Results:** We found increased levels of activated eosinophils in terminal ileum of patients with active IBD and IBD-PSC. All three activation markers were elevated in IBD, whereas only CD66b was elevated in IBD-PSC. The level of CXCR3 was elevated in IBD-PSC as well as the % of CD8<sup>+</sup> T-cells, indicating a Th1 type of inflammation in ileum. We found no obvious Th1 or Th2 profile in IBD without PSC in ileum or rectum. None of the activation markers were elevated in rectum in IBD-PSC, but eosinophils from patients with IBD had increased expression of CD69 and CD66b.

**Conclusions:** In line with our results, IBD-PSC patients often have a mild course of the intestinal inflammation which is mainly localized to the right colon, often causing back-wash-ileitis, with no inflammation in the rectum. Other studies suggest that PSC is a Th1 cytokine driven disease with a poor clinical responsiveness to glucocorticoid therapy, whereas IBD-patients often respond well to glucocorticoids. Our preliminary data seem to support these findings, and we conclude that the eosinophil activation phenotype in different intestinal diseases may depend on the predominant T-cell subtype in the intestine.

**POSTER 48****MOST EOSINOPHILS ARE UNDERGOING CYTOLYSIS IN EOSINOPHILIC ESOPHAGITIS**

Hedieh Saffari,<sup>1</sup> Laura Hoffman,<sup>2</sup> Kathryn A. Peterson,<sup>3</sup> Kristin M. Leiferman,<sup>4</sup> John C. Fang,<sup>3</sup> Leonard F. Pease III,<sup>1,3,5</sup> Gerald J. Gleich<sup>4,6</sup>

Departments of <sup>1</sup>. Chemical Engineering, <sup>2</sup>. Electron Microscopy Core Facility, <sup>3</sup>. Internal Medicine, Division of Gastroenterology, <sup>4</sup>. Dermatology, <sup>5</sup>. Pharmaceuticals and Pharmaceutical Chemistry, <sup>6</sup>. Internal Medicine, University of Utah, Salt Lake City, UT.

**Background:** In eosinophilic esophagitis (EoE), eosinophil granulocytes accumulate and release granule proteins onto epithelial tissues. However, little is understood about the mechanism of eosinophil degranulation.

**Aim:** To determine and quantify eosinophil degranulation patterns in biopsy specimens from EoE patients using transmission electron microscopy (TEM).

**Method:** Nine patients (3 female, 6 male) were randomly selected from those receiving care for EoE. Tissue specimens were obtained at endoscopy, fixed in glutaraldehyde, embedded in Epon, sectioned and imaged by TEM. Eosinophils and their granules were identified by their distinctive morphology, and all eosinophils and granules were imaged. A total of 18 tissue blocks from both proximal and distal esophageal specimens and 1672 images were graded. Each image was evaluated individually and independently by three reviewers (HS, LH and GJG). Eosinophils were categorized based on membrane integrity and the presence of piecemeal degranulation as judged by sombrero and/or other cytoplasmic vesicles. Granules were categorized based on whether they were associated with cells, free/extracellular and whether they showed reversal of staining/core lightening.

**Results:** More than 99% of eosinophils were abnormal with granules showing reversal of staining, evidence of piecemeal degranulation as judged by sombrero and/or other vesicles, and membrane disruption. Approximately 84% of eosinophils showed membrane disruption. Extracellular granules were abundant in at least 70% of the images and about 50% of these granules showed reversal of staining.

**Discussion:** Our findings indicate that almost all eosinophils infiltrating the esophagus in EoE demonstrate morphological abnormalities ranging from granule changes with reversal of staining to marked cytoplasmic vesiculation, particularly with sombrero vesicles, to loss of membrane integrity with cytolytic disruption and release of intact, membrane-bound granules into the tissues. Based on the prominent presence of sombrero vesicles, piecemeal degranulation appears closely related to the changes in eosinophil morphology in EoE. These findings indicate that eosinophils counted by light microscopy after routine H&E staining in EoE biopsies are likely abnormal with 84% showing frank cytolysis leading to release of intact granules onto esophageal tissues.

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## POSTER 49

**TREATMENT OF EOSINOPHILIC ESOPHAGITIS WITH THE CRTH2-ANTAGONIST OC000459: A NOVEL THERAPEUTIC PRINCIPLE**

A. Straumann<sup>1</sup>, S. Hoesli<sup>2</sup>, Ch. Bussmann<sup>3</sup>, M. Stuck<sup>2</sup>, M. Perkins<sup>4</sup>, L.P. Collins<sup>4</sup>, M. Payton<sup>4</sup>, R. Pettipher<sup>4</sup>, M. Hunter<sup>4</sup>, J. Steiner<sup>5</sup> & H.-U.Simon<sup>2</sup>

<sup>1</sup>Swiss EoE Clinic and EoE Research Network, Olten, Switzerland;

<sup>2</sup>Institute of Pharmacology, University of Bern, Bern, Switzerland;

<sup>3</sup>Institute of Pathology, General Hospital Lucerne, Lucerne, Switzerland;

<sup>4</sup>Oxagen Ltd., Milton Park, Abingdon, Oxon, United Kingdom;

<sup>5</sup>Oxford Therapeutics Consulting Ltd., Brightwell cum Sotwell, Oxon, United Kingdom

**Background:** Eosinophilic esophagitis (EoE) is a chronic, Th2-type inflammatory disease. CRTH2 (chemoattractant receptor-homologous molecule on Th2 cells) is a prostaglandin D2 (PGD2) receptor, expressed by Th2 cells and other inflammatory cells, including eosinophils and basophils, that mediates chemotaxis and activation. OC000459 is a selective CRTH2 antagonist and would be expected to suppress eosinophilic tissue inflammation.

The purpose of this study was to evaluate the efficacy and safety of an OC000459 monotherapy in adult patients with active, severe corticosteroid-dependent or corticosteroid-refractory EoE.

**Methods:** In this randomized, double-blind, placebo-controlled trial, 26 adult patients (m/f = 22/4; mean age 41 yrs, range 22-69 yrs) with active EoE, dependent or resistant to corticosteroids, were treated either with 100 mg OC000459 (n=14) or placebo (n=12) twice daily. Pre- and post-treatment disease activity was assessed clinically, endoscopically, histologically and via biomarkers. The primary endpoint was the reduction of esophageal eosinophil infiltration.

**Results:** After an 8-week OC000459 treatment, the esophageal eosinophil load decreased significantly, from 114.83 to 73.26 eosinophils per high power field (eos/hpf),  $p=0.0256$ ), whereas no reduction was observed with placebo (102.80 to 99.47 eos/hpf,  $p=0.870$ ). With OC000459, the physician's global assessment of disease activity improved from 7.13 to 5.18 ( $p=0.035$ ). OC000459 likewise reduced extracellular deposits of eosinophil peroxidase and tenascin C, effects not seen with placebo. No serious adverse events were observed.

**Conclusions:** An 8-week mono-therapy with the CRTH2-antagonist, OC000459, exerts modest, but significant anti-eosinophil and beneficial clinical effects in adult patients with active, severe, corticosteroid-dependent or corticosteroid-refractory EoE, and is well tolerated.

## POSTER 50

**THE ESOPHAGEAL STRING TEST QUANTIFIES LUMINAL EOSINOPHIL AND CHEMOKINE BIOMARKERS REFLECTIVE OF ESOPHAGEAL INFLAMMATION IN ADULTS WITH EOSINOPHILIC ESOPHAGITIS**

Steven J. Ackerman,<sup>1</sup> Nirmala Gonsalves,<sup>2</sup> Preeth Alumkal,<sup>1</sup> Jian Du,<sup>1</sup> Brian T. Maybruck,<sup>1</sup> Christine E. Nelson,<sup>2</sup> Angelika Zalewski,<sup>2</sup> Guang-Yu Yang,<sup>3</sup> Wendy Moore,<sup>4</sup> Rachel Harris,<sup>4</sup> Glenn T. Furuta<sup>4</sup> and Ikuo Hirano<sup>2</sup>

<sup>1</sup>Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, IL, <sup>2</sup>Department of Medicine and <sup>3</sup>Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL, and <sup>4</sup>Gastrointestinal Eosinophilic Diseases Program, Section of Pediatric Gastroenterology, Hepatology and Nutrition, Mucosal Inflammation Program, University of Colorado School of Medicine, Aurora, CO, USA

**Background:** Eosinophilic Esophagitis (EoE) is a chronic disease in both children and adults characterized by eosinophil-predominant esophageal inflammation. Endoscopy (EGD) with biopsy is the current standard to assess mucosal inflammation and follow response to therapy. We recently reported that eosinophil-derived granule proteins (EDGPs) could be quantified in esophageal secretions in children using a minimally invasive Esophageal String Test (EST) that can distinguish active EoE from treated EoE in remission, GERD and normal esophagus (1).

**Aims:** To determine whether the EST can assess disease activity using esophageal luminal biomarkers of eosinophil recruitment and inflammation in adults with EoE.

**Methods:** Ten adults (mean age 43.7; range 34-61; 6 male, 4 female) with EoE who underwent EGD for either diagnosis or evaluation of responses to treatment performed overnight 10-12 hour ESTs prior to their scheduled EGD. The Enterotest™ string was removed before EGD, and the proximal and distal esophageal segments were processed to elute the luminal effluent on the string (1). EDGPs: major basic protein-1 (MBP1), eosinophil-derived neurotoxin (EDN) and Charcot-Leyden Crystal Protein/Galectin-10 (CLC/Gal-10), and chemoattractants (Eotaxin-2, Eotaxin-3) were measured by ELISA (1) in the EST samples (n=10).

**Results:** In patients with active EoE ( $\geq 15$  eos/HPF, n=7), proximal/distal eosinophil counts ranged from 10/25 to  $>100/>100$ ; in treated patients in remission ( $<15$  eos/HPF, n=3), eosinophil counts ranged from 0/0 to 10/3. In active EoE, EST measurement of EDGP biomarkers showed increased MBP1 (12,745 $\pm$ 2,538 ng/ml), EDN (2,497 $\pm$ 931 ng/ml) and CLC/Gal-10 (895 $\pm$ 287 ng/ml), whereas patients in remission had lower levels of these biomarkers [MBP1 (5,446 ng/ml), EDN (882 $\pm$ 157 ng/ml), CLC/Gal-10 (288 $\pm$ 116 ng/ml)]. Similarly, in active EoE, the EST captured increased eotaxin-2 (4,696 $\pm$ 1602 pg/ml) and eotaxin-3 (1,029 $\pm$ 378 pg/ml) compared to treated patients in remission [eotaxin-2 (2,383 $\pm$ 443 pg/ml) and eotaxin-3 (377 $\pm$ 19 pg/ml)]. The levels of the EDGPs in distal EST samples were correlated with distal eosinophil counts (EDN,  $r=0.637$ ,  $p<0.05$ ; CLC/Gal-10,  $r=0.571$ ,  $p=0.085$ ) analyzed for all 10 EoE subjects. Similarly, EST levels of eotaxin-2 and eotaxin-3 were correlated with eosinophil counts (eotaxin-2,  $r=0.614$ ,  $p=0.059$ ; eotaxin-3,  $r=0.797$ ,  $p<0.01$ ) analyzed for all 10 EoE subjects.

**Conclusions:** The EST captures eosinophil-associated biomarkers and eosinophil-selective chemoattractants in adults with EoE in an overnight sampling, and levels of these biomarkers correlate with the numbers of eosinophils in esophageal biopsies, the current standard for EoE activity. Thus the EST is a minimally invasive method capable of quantifying esophageal eosinophilic inflammation in adults with EoE as a measure of disease activity.

**Reference:** (1) Furuta GT et al and Ackerman SJ. The oesophageal string test: a novel, minimally invasive method measures mucosal inflammation in eosinophilic oesophagitis. Gut. 2012 Aug 15 [Epub ahead of print] PMID: 22895393.

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## POSTER 51

**REGULATION OF BLOOD EOSINOPHIL PHENOTYPES BY TOPICAL CORTICOSTEROIDS IN PATIENTS WITH EOSINOPHILIC ESOPHAGITIS**Lingblom Christine <sup>1\*</sup>, Bergquist Henrik <sup>2\*</sup>, Johnsson Marianne <sup>1</sup>, Bove Mogens <sup>3</sup> & Wennerås Christine <sup>1,4</sup><sup>1</sup>Department of Infectious Diseases, Sahlgrenska Academy, Gothenburg University, Sweden<sup>2</sup>Department of ENT, Head and Neck Surgery, Sahlgrenska Academy, Gothenburg University, Sweden<sup>3</sup>Department of ENT, Head and Neck Surgery, NÄL Hospital, Trollhättan, Sweden<sup>4</sup>Department of Hematology and Coagulation, Sahlgrenska Academy, Gothenburg University, Sweden

Eosinophilic esophagitis (EoE) is a chronic antigen-driven inflammatory disease where eosinophils invade the mucosa of the esophagus, a part of the gastrointestinal tract that is usually devoid of these cells. The reason why EoE occurs is still uncertain but allergens have been implicated. Our group has previously shown that blood eosinophils from EoE patients have a particular phenotype. Adult EoE patients are often treated with topical corticosteroids. The aim of this study was to examine if topical corticosteroids could normalize the eosinophilic phenotype of EoE patients to a healthy one. Blood eosinophils from adult EoE patients ( $n=13$ ) were examined before and after treatment with topical corticosteroids ( $n=7$ ) and compared to healthy controls ( $n=10$ ). The patients were given Mometasone furoate ( $200\mu\text{g}$  4 times daily for 2 months). Eight chosen surface markers on eosinophils were analyzed with four-color flow cytometry. The different molecules were the chemokine receptor CCR3, the adhesion molecule CD54, the prostaglandin D2 receptor CRTH2, the low-affinity IgE receptor CD23, the receptor for hyaluronic acid CD44, the integrin CD18, the counter-ligand of CD40L and formyl peptide receptors. EoE patients had decreased expression of CD44 and CCR3 and increased expression of CD23, CD40 and CD54 compared to healthy controls. Corticosteroids decreased the level of CD18 expression but did not otherwise alter the phenotype of eosinophils. The expression of CD18 and FPR correlated positively with eosinophil numbers in the esophagus at the time of diagnosis and a negative association was seen. To summarize, topical corticosteroids had a limited effect on the phenotype of eosinophils in EoE, restricted to lowering surface expression of CD18. CD18 is an adhesion molecule, this may explain the reduced numbers of eosinophils in the esophagus of corticosteroid-treated EoE patients.

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## POSTER 52

**THE EOSINOPHIL CATIONIC PROTEIN, AN EOSINOPHIL GRANULE PROTEIN EFFECTIVE AGAINST MYCOBACTERIA**David Pulido<sup>1</sup>, Marc Torrent<sup>1,2</sup>, M. Moussaoui<sup>1</sup>, David Andreu<sup>3</sup>, M. Victoria Nogués<sup>1</sup> and Ester Boix<sup>1</sup><sup>1</sup>Department of Biochemistry and Molecular Biology, Biosciences Faculty, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain<sup>2</sup>Regulatory Genomics and Systems Biology, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom<sup>3</sup>Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, Barcelona, Spain

**Background:** There is an urgent need to develop new agents against mycobacterial infections, such as tuberculosis and other respiratory tract or skin affections. When eosinophilia was first linked to tuberculosis, eosinophils were regarded as mere offenders, exacerbating pulmonary inflammation. Notwithstanding, later bibliography evidenced their protective role contributing to bacterial clearance at the infection focus. Eosinophils are recruited in lung granulomas, releasing their granule content into macrophages, where they can target intracellular pathogens. Eosinophil granule proteins are therefore suitable weapons to eradicate the macrophage resident bacteria. As eosinophils were reported to be activated by mycobacterial infection and the Eosinophil Cationic Protein (ECP) was previously found to contribute to mycobacterial growth inhibition<sup>1</sup> we committed ourselves to investigate the protein potential toxicity against mycobacteria.

**Methods:** Recombinant ECP was expressed in *E. coli* and purified as previously described<sup>2</sup>. Peptide ECP(1-45) was synthesized and purified as previously<sup>2</sup>. *Mycobacterium vaccae* was chosen as a non-pathogenic working specie model. Bacteria viability was assayed using the *Bact-Titer Glo* and *LIVE/DEAD* kits. Minimal inhibitory concentration (MIC) and minimal agglutination concentration (MAC) were calculated as previously<sup>3</sup>. Membrane permeation and depolarization were assayed by the *Sytox Green* and the DiSC3(5) fluorescent assays respectively. Transmission and scanning electron microscopies (TEM and SEM) were applied to visualize the damage at the mycobacteria cell envelope.

**Results:** ECP has been proven effective against *Mycobacterium vaccae* at a low micromolar level. High bactericidal activity correlated with its bacteria membrane depolarization and permeabilization activities. Further analysis identified an active N-terminus derived peptide that retained the antimicrobial and bacteria agglutinating activities. Electron microscopy confirmed the protein and peptide damage at the cell envelope. SEM micrographies were also evaluated to estimate the size and density of bacteria aggregates.

**Conclusions:** ECP and the ECP(1-45) N-terminal peptide display high antimycobacterial and cell agglutination activities. The results support the contribution of secretion granule proteins to the host immune response against mycobacteria. We hypothesize that the eosinophil secretion protein may target *in vivo* the mycobacteria dwelling inside macrophages, promoting the clearance of macrophage resident bacteria. Human-derived antimicrobial peptides showing high targeted cytotoxicity but low immunogenicity are promising antimycobacterial therapeutic agents. The particular biophysical properties of the ECP active peptide are envisaged as a suitable reference for the development of novel antimycobacterial drugs.

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## POSTER 53

**GLYCOSYLATION MODULATES THE ANTIMICROBIAL ACTIVITY OF HUMAN EOSINOPHIL CATIONIC PROTEIN NATIVE FORMS**

Vivian A. Salazar<sup>1</sup>, Jenny Rubin<sup>2</sup>, David Pulido<sup>1</sup>, Mohammed Moussaoui<sup>1</sup>, Per Venge<sup>2</sup> and Ester Boix<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Fac. Biosciences, Universitat Autònoma de Barcelona, Spain

<sup>2</sup>Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden

**Background:** The Eosinophil Cationic Protein (ECP) is a granule protein secreted by eosinophils during inflammation and infection. The protein cytotoxicity can target a wide variety of pathogens. Previous work identified in human eosinophil extracts several ECP native variants with different degrees of glycosylation (1) that modify the protein biological properties (2, 3). In this study we have analyzed the contribution of the posttranslational modifications on the protein antimicrobial activity.

**Methods:** Low and heavy glycosylated ECP forms were purified from eosinophil granules extracts. Buffy coats of healthy blood donors were processed as previously described (1). Bactericidal and cell agglutination activities were assayed as described (4). Bacteria cell leakage was assessed by Sytox Green release and direct membrane leakage was assayed by detection of liposomes encapsulated markers of distinct molecular weights. Lipopolysaccharide binding was calculated using a fluorescent probe by an occupancy displacement assay.

**Results:** Native ECP forms were tested on *E. coli* cell cultures and compared to the recombinant non-glycosylated protein. Further analysis on model membranes provided an insight towards the understanding of the protein mechanism at the cytoplasmic membrane. Glycosylation mostly hinders the protein bacteria agglutination activity and slightly reduces its interaction with the outer membrane lipopolysaccharides. Notwithstanding, the lower glycosylated fraction mostly retains the ability to trigger the membrane leakage on lipid bilayers and induce the cell membrane depolarization. Structural analysis suggest that glycosylation may interfere with the protein self aggregation process at the lipid bilayer altering the membrane disruption process. However, the protein cytotoxicity is mostly retained by the low glycosylated forms.

**Conclusions:** We can conclude that while heavy glycosylation drastically reduces the protein toxicity on bacteria cells, low glycosylation do not interfere with the main protein antimicrobial properties. Experimental data on a membrane model reveal a distinct membrane permeabilization strategy for native ECP respect to its recombinant counterpart. The results indicate that glycosylation modulates the protein mechanism of action at the lipid bilayer and bacteria wall level. Work is in progress to elucidate the physiological role of the protein posttranslational modifications associated to the host immune response and in particular to the protein targeting of invading pathogens.

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## POSTER 54

**EOSINOPHILS: AN UNFORESEEN PROTAGONIST IN INFLUENZA INFECTION**

Amali E. Samarasinghe<sup>1,3</sup>, Stacie N. Woolard<sup>2,3</sup>, and Jonathan A. McCullers<sup>1,3</sup>

<sup>1</sup>Department of Infectious Diseases and <sup>2</sup>Department of Tumor Cell Biology, <sup>3</sup>St Jude Children's Research Hospital

**Background:** The global incidence of asthma is expected to increase by 100 million in the next 15 years. Respiratory viral infections have long been implicated in asthma induction and exacerbation. During the 2009 influenza pandemic, the importance of influenza virus in the context of allergic asthma was highlighted by the strong link to hospital admissions. However, outcomes in this cohort are not yet clear. Understanding the overlap between these two diseases is crucial in order to develop therapeutics to protect the asthma population from viral infections. Using a co-morbidity model of asthma and influenza to characterize hallmarks of both acute and chronic asthma, we have found that mice with characteristics of acute asthma are protected from influenza-induced morbidity. Our objective for this study was to identify the mechanism that mediated protection in mice with acute asthma and viral disease.

**Methods:** *Aspergillus fumigatus* was used as the triggering allergen to induce allergic airway inflammation and remodeling in age-matched female C57BL/6 mice. Eosinophils were harvested from these mice and adoptively transferred to animals that were infected intranasally with 1000 TCID<sub>50</sub>/ml A/CA/04/2009 H1N1 pandemic strain of influenza (pH1N1). Non-allergic mice infected with virus served as negative controls, while allergic mice infected with pH1N1 served as positive controls. Body weight, lung viral titers, antibodies, cytokines, lung mRNA expression, and histological analyses were performed to determine whether eosinophils played a role in mediating protection against influenza.

**Results:** Mice that received eosinophils directly into the airways lost equivalent weight compared to the negative controls. However, these mice had a significant reduction in viral burden and a large influx of CD8<sup>+</sup> T cells. Eosinophil transfer caused changes to the cytokine profile in the bronchoalveolar lavage fluid. Chronic asthmatic mice that were supplemented with eosinophils recovered sooner than controls and also had reduced viral titers in the lungs. Furthermore, the integrity of the airway columnar epithelium was maintained in virus infected mice that received eosinophils.

**Conclusions:** Eosinophils mediate protection against influenza possibly by promoting anti-viral defenses of the airway structural cells. These initial studies provide an impetus to investigate the underlying mechanisms that mediate altered outcomes during influenza in acute inflammation of allergic airways. Ongoing studies are aimed at identifying specific changes in the eosinophil phenotype in relation to the airway epithelium following influenza.

**Grant support:** *This study was supported by public health service grant 1 N01 AI-70005-C (McCullers).*

## POSTER 55

THE ROLE OF EOSINOPHILS IN IMMUNITY TO THE TREMATODE HELMINTH *SCHISTOSOMA HAEMATOBIIUM*

Shona Wilson<sup>1</sup>, Frances M Jones<sup>1</sup>, Hassan KM Fofana<sup>2</sup>, Aissata Doucouré<sup>2</sup>, Aly Landouré<sup>2</sup>, Gachuhi Kimani<sup>3</sup>, Joseph K Mwatha<sup>3</sup>, Moussa Sacko<sup>2</sup>, Birgitte J Vennervald<sup>4</sup>, David W Dunne<sup>1</sup>

<sup>1</sup>Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, UK

<sup>2</sup>Institut National de Recherche en Santé Publique, Bamako, BP 1771, Mali

<sup>3</sup>Kenya Medical Research Institute, Nairobi, Kenya

<sup>4</sup>DBL – Centre for Health Research and Development, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 57, 1870 Frederiksberg C, Denmark

**Background:** Pre-treatment eosinophil number and IgE specific to worm antigen (SWA) are associated with human immunity to re-infection with schistosomes after chemotherapeutic treatment. Treatment significantly elevates circulating IL-5 24-hr post-treatment of *Schistosoma mansoni*.

**Methods:** The relationship between pre-treatment eosinophil number, SWA-IgE, and infection intensity and the 24-hr post-treatment IL-5 boost was investigated in a Malian cohort (n=326, aged 5-40yrs), exposed to *S. haematobium*. Eotaxin levels were measured at 24-hr post-treatment as a proxy of eosinophil migration. The relationship between the 24-hr post-treatment IL-5 boost and later eosinophil numbers and SWA-IgE levels (9-wk post-treatment) was examined, then investigated in the context of subsequent re-infection (2-yr post-treatment).

**Results:** Circulating IL-5 levels increased 24-hr post-treatment and were associated with pre-treatment infection intensity ( $\hat{\alpha}=0.071$ ,  $p<0.05$ ), SWA-IgE levels ( $\hat{\alpha}=0.189$ ,  $p<0.001$ ), eosinophil number ( $\hat{\alpha}=0.418$ ,  $p<0.001$ ), as well as 24-hr post-treatment eotaxin levels ( $\hat{\alpha}=0.333$ ,  $p<0.001$ ). Twenty-four hr IL-5 levels were, in turn, significantly associated with eosinophil number ( $\hat{\alpha}=0.121$ ,  $p<0.001$ ) and elevated SWA-IgE 9-wk later ( $\hat{\alpha}=0.085$ ,  $p<0.05$ ). These SWA-IgE levels were significantly associated with immunity to re-infection (OR=0.706,  $p<0.05$ ).

**Conclusions:** Early IL-5 production after treatment-induced exposure to *S. haematobium* worm antigen is positively associated with antigen dose (infection intensity), IgE availability for arming of effector cells at time of treatment and subsequent eosinophil migration. The IL-5 produced is positively associated with increased later eosinophil number and increases in specific IgE levels. This implicates eosinophils, and their role in the IL-5 boost, in down-stream responses that lead to subsequent re-infection immunity.

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## POSTER 56

**TH-MRSVG, MODIFIED RSVG PROTEIN FROM RSV A2, INDUCES PROTECTION AGAINST RSV WITHOUT IMMUNOPATHOGENESIS**

In Su Cheon<sup>1,2†</sup>, Byoung-Shik Shim<sup>2†</sup>, Sung Moo Park<sup>1</sup>, Ji Eun Jang<sup>2</sup>, Dae Im Jung<sup>2</sup>, Jun Jang<sup>3</sup>, Manki Song<sup>2</sup> and Cheol-Heui Yun<sup>1</sup>

<sup>1</sup>Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea, <sup>2</sup>Laboratory Sciences Division, International Vaccine Institute, Seoul, Korea, <sup>3</sup>Division of Life & Pharmaceutical Sciences Ewha Womans University School of Medicine, Seoul, Korea

**Background:** Respiratory syncytial virus (RSV) is a major cause of respiratory tract infections and diseases in infants and young children worldwide. Although almost all children are infected with the virus by 2 to 3 years of age, currently there is no effective vaccine available. Formalin-inactivated RSV, tried as a clinical RSV vaccine in 1960s, exacerbated pulmonary disease including imbalanced Th2-biased immune inflammatory response and eosinophil recruitment into the lung. The G glycoprotein of RSV (RSVG), a major attachment protein, is a potentially important target for protective anti-viral immune responses. In addition, it has been known that a CD4<sup>+</sup> T cell epitope (aa183-195) of G protein is closely related to the induction of eosinophilia. Here, we investigated whether new G protein (Th-mRSVG) provides protection against RSV without vaccine-induced-immunopathology.

**Methods:** RSVG, mRSVG or Th-mRSVG protein derived from RSV A2 strain were expressed in *E. coli* and purified to homogeneity. Balb/c mice were immunized with various RSVG proteins plus cholera toxin (CT) two times at 2-week intervals via sublingual route (s.l.) and then challenged with live RSV. After 4 days, eosinophils in the lung and viral clearance were measured by flow cytometry or plaque assay, respectively. The change of body weight was monitored every day. The level of antigen-specific antibody (Ab) was measured in serum and BAL.

**Results:** To develop a non-pathogenic and effective RSV vaccine, two amino acids from this CD4 epitope were changed (mRSVG) and a CD4 epitope of RSV F protein was fused to this mutant RSV G core fragment (aa131-230). The level of Ab was higher in mouse immunized with Th-mRSVG than that in mRSVG. The s.l. administration of Th-mRSVG and wild type RSVG (wtRSVG) provided a protection against RSV A2 challenge with partial cross protection against B type RSV challenge. However, mice immunized with wtRSVG showed immunopathogenesis including eosinophils recruitment into lung and body weight loss, as reported previously whereas in mice immunized with Th-mRSVG or mRSVG, immunopathogenesis especially eosinophilia was not observed. Moreover, it was apparent that CD4 epitope of G protein is important to induce eosinophilia into the lung because the infiltration of eosinophils was reduced in mice administrated with mRSVG or Th-mRSVG.

**Conclusion:** The s.l. delivery of Th-mRSVG provided protective immunity against RSV infection without causing any vaccine-induced immunopathology such as eosinophilia or body weight loss. Thus, our study could offer a novel strategy to develop a safe and effective RSV vaccine.

## POSTER 57

**EOSINOPHILS INFLUENCE THE INFLAMMATORY PHENOTYPE OF ASTHMA IN AN INDUCIBLE KNOCK-IN EOSINOPHIL-DEFICIENT MOUSE MODEL OF ASTHMA.**

Elizabeth A. Jacobsen<sup>1</sup>, William E. LeSuer<sup>1</sup>, Lian Willetts<sup>1,2,3</sup>, Nathalie Antonios<sup>1</sup>, Cheryl Protheroe<sup>4</sup>, Sergei I. Ochkur<sup>1</sup>, Dana Colbert<sup>4</sup>, Nancy A. Lee<sup>4</sup>, and James J. Lee.<sup>1</sup>

<sup>1</sup> Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

<sup>2</sup> Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, AB, T6G 2S2

<sup>3</sup> Department of Immunology, University of Manitoba, Winnipeg, MB, and Division of Allergy and Immunology, R<sup>3</sup>E<sup>0</sup>W<sup>2</sup>

<sup>4</sup> Divisions of Haematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

**Background:** Although eosinophils are a predominant cellular infiltrate in mouse models of allergic asthma and often associated with clinical symptoms in patients, the role of these cells remains to be defined. Recently eosinophils have been demonstrated to have Th2 immune modulating activities in asthma. Consequently, asthma is often categorized into Th2 eosinophilic or Th1/Th17 neutrophilic phenotypes in patients, suggesting separate development and progression of the disease. The ability to ablate eosinophils in an otherwise normal animal enables the opportunity to determine causative effects of eosinophil immune modulation on asthma phenotypic sub-types.

**Objective:** To demonstrate a role for eosinophils in immune modulation of asthma phenotype using an inducible knock-in mouse model.

**Methods:** The knock-in inducible eosinophil-less mice (iPHIL) express diphtheria toxin (DT) receptor (DTR) from the eosinophil peroxidase (EPX) promoter, which enables eosinophil-specific targeted cell death. To determine eosinophil blood, bone marrow, lymphatic, and tissue kinetics at homeostatic baseline, iPHIL and wild type (C57BL/6 background) mice were injected with 15ng/gm body weight of DT for two consecutive days or extended periods up to 28 days. Assessments were made by DiffQuick staining and differential counts, flow cytometry, and anti-major basic protein immunohistochemistry. For mouse models of asthma, mice were sensitized and challenged with established acute OVA or HDM allergen protocols. Depletion of eosinophils was completed during either sensitization or challenge with allergen. Measurements of bronchoalveolar (BAL) cellularity, differentials, cytokines, and lung histopathologies were assessed.

**Results:** Administration of DT was successful in depleting eosinophils from blood, bone marrow, and lymphatics within 4 to 5 days. Longer administration of DT was required to deplete tissue eosinophils (i.e., gastrointestinal and uterus), suggesting a slower turnover rate for tissue eosinophils. No other cells are affected by DT administration. Administration of DT during the sensitization of OVA/Alum (i.p) or HDM (i.n.) did not alter the Th2 immune phenotype of the allergic response. Conversely, administration of DT during challenge of the OVA and HDM protocol resulted in two distinct inflammatory phenotypes. The depletion of eosinophils during challenge either resulted in 1) non-inflammatory response (i.e., no eosinophils and no neutrophils) ( $\emptyset$ -iPHIL) or 2) non-eosinophilic and high neutrophilic (neu-iPHIL) phenotype. This neu-iPHIL phenotype resulted in histopathological changes such as increased cellularity and increased mucus production/goblet metaplasia.

**Conclusion:** We successfully generated a novel mouse model (iPHIL) that enables inducible and specific ablation of eosinophils in circulation, bone marrow, and tissues. This data shows that the presence of eosinophils is likely to be an important modulator of the sub-type of asthma, particularly during the challenge (i.e., secondary immune) responses of asthma. For example, depletion of eosinophils during allergen challenge, regardless of allergen or delivery method, resulted in a both a non-inflammatory phenotype ( $\emptyset$ -iPHIL) and a neutrophilic phenotype (neu-iPHIL). This suggests a role for eosinophils in suppressing the neutrophilic during allergen challenges in mouse models of asthma. Furthermore, our studies indicate a possible unappreciated function of eosinophils in various sub-types of asthma such as neutrophilic asthma in patients.

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

## POSTER 58

**IMPAIRED AIRWAY EOSINOPHIL MIGRATION INTO THE PARATRACHEAL LYMPH NODES IN LTC4 SYNTHASE-DEFICIENT MICE**Haibin Wang

**Background:** We previously demonstrated in mice that airway eosinophils, including those instilled intratracheally, can traffic from the airway lumen into lung-draining paratracheal lymph nodes and function as antigen-presenting cells to elicit primary and secondary T cell responses to inhaled antigens. However, the mechanisms whereby airway eosinophils traverse from the lung and home to paratracheal lymph nodes are still unclear. We investigated the roles of cysteinyl leukotrienes in mediating eosinophil trafficking from the lungs to paratracheal lymph nodes.

**Methods:** We studied the effect of cysteinyl leukotrienes on airway eosinophil trafficking by using LTC4 synthase-deficient (LTC4S<sup>-/-</sup>) mice, which due to the lack of this enzyme are incapable of synthesizing LTC4. Eosinophils from the spleens of IL-5 transgenic mice, labeled *ex vivo* with a fluorescent red dye-DiI16(3), were intratracheally injected into OVA-sensitized and OVA-aerosol challenged WT (LTC4S<sup>+/+</sup>) or LTC4S<sup>-/-</sup> mice before the last OVA-aerosol challenge. Twenty-four hours after injection, the paratracheal lymph nodes and lungs were harvested, and the fluorescently-labeled donor eosinophils and host recipient-derived eosinophils (SSC<sup>high</sup>/Siglec-F<sup>+</sup>) were identified by fluorescence microscopy and flow cytometry. In some experiments, mice received intratracheal exogenous LTC4 one day before eosinophil injection. Transwell assays were used to test chemotactic responses of LTC4S<sup>+/+</sup> or LTC4S<sup>-/-</sup> eosinophils, purified from bronchoalveolar lavage fluid of OVA-sensitized and OVA-aerosol challenged LTC4S<sup>+/+</sup> or LTC4S<sup>-/-</sup> mice, to CCL19 *ex vivo*. The expressions of CCR7 and cysLT receptors by eosinophils were determined by FACS and RT-PCR, respectively.

**Results:** We observed significantly diminished migrations into paratracheal lymph nodes of both intratracheally introduced donor and host recipient eosinophils in inflamed lungs of LTC4S<sup>-/-</sup> mice, and that eosinophils were less capable of exiting the inflamed lungs in LTC4S<sup>-/-</sup> mice. The impaired eosinophil migration was partially restored by exogenous LTC4 *in vivo*, and completely reversed *ex vivo* if LTC4S<sup>-/-</sup> eosinophils were pretreated with LTC4 before their chemotactic response to CCL19, indicating that optimal chemotaxis to CCL19 requires triggering from CysLTs. We also demonstrated that mouse eosinophils constitutively express the CCL19 binding receptor CCR7 and contain mRNA transcripts for both known cysLT1 and cysLT2 receptors, which might mediate cysteinyl leukotriene-directed eosinophil migration.

**Conclusion:** Our findings that cysteinyl leukotrienes are involved in regulating airway and lung eosinophil migration to paratracheal lymph nodes provide a previously unrecognized role for the cysteinyl leukotrienes, 5-lipxygenase-derived eicosanoid mediators, in regulating the pulmonary trafficking of eosinophils in experimental allergic asthma.

**Grant support:** Funded by NIH grants R37-AI020241 and R01-AI051645 (PFW)

## POSTER 59

**A MOUSE WITH EFFICIENT EXPRESSION OF CRE-RECOMBINASE EXCLUSIVELY IN EOSINOPHILS**

Alfred D. Doyle<sup>1</sup>, Elizabeth A. Jacobsen<sup>1</sup>, Sergei I. Ochkur<sup>1,2</sup>, Lian Willets<sup>1,3</sup>, Kelly Shim<sup>1</sup>, Joseph Neely<sup>2</sup>, Jake Kloeber<sup>1</sup>, William E. LeSuer<sup>1</sup>, Ralph S. Pero<sup>1</sup>, Paige Lacy<sup>3</sup>, Redwan Moqbel<sup>4</sup>, Nancy A. Lee<sup>2</sup>, and James J. Lee<sup>1</sup>

<sup>1</sup>Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ; <sup>2</sup>Division of Hematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ; <sup>3</sup>Pulmonary Research Group, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; <sup>4</sup>Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada

**Background/Objective:** The inability to target gene expression in eosinophils (either positively or negatively) has remained a significant obstacle to mechanistic studies of eosinophils effector functions and the roles of these leukocytes in health and disease. We have generated an eosinophil specific *Cre*-recombinase expressing strain of mice, eoCRE, as a logistical solution to this obstacle and to facilitate a better understanding of eosinophil-mediated activities.

**Methods:** We employed homologous recombination in embryonic stem cells to knock-in a mammalianized *Cre*-recombinase at the AUG start codon of the open-reading frame encoding the Eosinophil Peroxidase (EPX) gene. The resulting mice (eoCRE) were assessed for levels of eosinophils in the peripheral blood by WBC counts and differentials based on blood smears and by flow cytometric assessments. *Ex vivo* marrow culture was performed to assess for *Cre* toxicity. eoCRE mice were crossed with loxP-stop-loxP GFP reporter mice to assess efficiency and specificity of *Cre* expression and peripheral blood and marrow was examined by flow cytometry. Finally, eoCRE mice were crossed with loxP-stop-loxP Diphtheria Toxin A (DTA) mice to further assess the ability of this model system to mediate eosinophil-specific gene expression.

**Results:** Our studies crossing eoCRE with loxP-stop-loxP GFP reporter mice demonstrated that our knock-in strategy at the endogenous EPX locus created a strain of mice with functionally significant *Cre* expression (i.e., ability to mediate loxP site-specific recombination) in greater than 95% ( $96.5 \pm 1.4\%$ ) of peripheral blood eosinophils. More importantly, flow cytometric assessments determined that this *Cre*-expression was absolutely eosinophil-specific with  $99.5 \pm 0.3\%$  of peripheral blood leukocytes expressing the GFP reporter gene also displaying eosinophil-specific cell surface markers (CCR3+, IL-5R $\alpha$ +). Finally, we crossed the eoCRE mouse with a loxP-stop-loxP Diphtheria Toxin A (DTA) mouse and found that the resulting mice (eoCRE/DTA) had an eosinophil deficiency equivalent to a previously reported "gold standard" eosinophil-deficient strain of mice (i.e., PHIL).

**Conclusions:** eoCRE mice display normal levels of blood and marrow eosinophils with no evidence of *Cre* toxicity. Expression of *Cre* occurs exclusively in eosinophils and at a level that mediates recombination of reporter genes in >95% of all circulating and tissue resident eosinophils. In addition, eoCRE mice crossed with a loxP-stop-loxP DTA strain generated a novel line of mice congenitally deficient of eosinophils. Collectively, these results establish a highly efficient eosinophil-specific *Cre* mouse that will be instrumental in defining the activities of the eosinophil in health and disease.

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## POSTER 60

**MICE DEFICIENT IN THE  $\alpha$ 2,3 SIALYLTRANSFERASE ST3GAL-III SELECTIVELY MANIFEST ENHANCED ALLERGIC EOSINOPHILIC AIRWAY INFLAMMATION**

Takumi Kiwamoto<sup>1</sup>, Mary E. Brummet<sup>1</sup>, Fan Wu<sup>1</sup>, Mary G. Motari<sup>2</sup>, Ronald L. Schnaar<sup>2</sup>, Zhou Zhu<sup>1</sup>, Bruce S. Bochner<sup>1</sup>

<sup>1</sup> Department of Medicine, Division of Allergy and Clinical Immunology and <sup>2</sup> Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21224, USA

**Background:** Siglec-F is a pro-apoptotic receptor on mouse eosinophils, but little is known about its natural tissue ligand.

**Objective:** We previously reported that the  $\alpha$ 2,3 sialyltransferase ST3gal-III (*St3gal3* gene product) is required for constitutive Siglec-F lung ligand synthesis (Guo et al. Am. J. Respir. Cell Mol. Biol. 44: 238-243, 2011). We therefore hypothesized that attenuation of ST3gal-III will decrease Siglec-F ligand levels and enhance allergic eosinophilic airway inflammation.

**Methods:** C57BL/6 wild type (WT) mice and *St3gal3* heterozygous or homozygous deficient (*St3gal3*<sup>+/-</sup> and *St3gal3*<sup>-/-</sup>) mice were used. Eosinophilic airway inflammation was induced via ovalbumin (OVA) sensitization and repeated intranasal OVA challenge. Siglec-F human IgG1 fusion protein (Siglec-F-Fc) was used to detect Siglec-F ligands. Lung tissue and lavage fluid (BALF) were analyzed for various cytokines and chemokines. Serum was analyzed for allergen-specific immunoglobulin levels.

**Results:** Western blotting of normal mouse whole lung extracts with Siglec-F-Fc detected sialidase-sensitive  $\approx$ 500kDa and  $\approx$ 200kDa candidate Siglec-F ligands that were less abundant in *St3gal3*<sup>+/-</sup> lung extracts and absent or nearly absent in *St3gal3*<sup>-/-</sup> lung extracts. Immunohistochemistry determined that sialidase-sensitive candidate Siglec-F ligands were expressed in airway epithelium and tracheal submucosal glands. Surprisingly, the Siglec-F ligands were still present in the submucosal glands in *St3gal3*<sup>-/-</sup> mice. After OVA sensitization and challenge, Siglec-F ligands were increased in wild type mouse lungs but less so in *St3gal3* mutants whereas there were marked and selective increases in peribronchial and BALF eosinophils seen with rank order *St3gal3*<sup>-/-</sup>  $\geq$  *St3gal3*<sup>+/-</sup> > WT mice. Levels of various cytokines and chemokines in BALF were not significantly different between these three types of mice, although OVA-specific serum IgG1 was increased in *St3gal3*<sup>-/-</sup> mice. Circulating blood levels of eosinophils were not increased in *St3gal3*<sup>-/-</sup> or *St3gal3*<sup>+/-</sup> mice compared to WT mice

**Conclusions:** After OVA sensitization and challenge, *St3gal3*<sup>+/-</sup> and *St3gal3*<sup>-/-</sup> mice have more intense allergic eosinophilic airway inflammation and less sialylated Siglec-F ligands in their airways. These data suggest that reducing levels of airway ligands for Siglec-F may diminish a natural pro-apoptotic pathway for controlling airway eosinophilia.

**Grant support:** This work was supported by grants AI72265 and HL107151 from the National Institutes of Health.

## POSTER 61

**A NOVEL METHOD TO EXPLORE EOSINOPHIL *IN VIVO* TRAFFICKING IN THE MOUSE**

Eva M. Sturm<sup>1,2</sup>, Kimberly D. Dyer<sup>1</sup>, Caroline M. Percopo<sup>1</sup>, Gunter J. Sturm<sup>3</sup>, Akos Heinemann<sup>2</sup>, Helene F. Rosenberg<sup>1</sup>

<sup>1</sup> Inflammation and Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

<sup>2</sup> Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

<sup>3</sup> Department of Dermatology and Venereology, Division of Environmental Dermatology and Venereology, Medical University of Graz, Austria

**Background:** Here we describe a novel method via which *ex vivo* cultured mouse bone marrow-derived eosinophils (bmEos) can be adoptively transferred into recipient mice in order to study receptor-dependent recruitment to lung tissue *in vivo*.

**Methods:** Bone marrow cells were collected from Balb/c donor mice and differentiated into mature eosinophils using an *ex vivo* cell culture system. Mature bone marrow-derived eosinophils (bmEos) were collected and transferred into wild type mice or eosinophil-deficient *ΔdblGATA* mice via tail vein injection. Prior to the cell transfer, recipient mice were either left untreated or were anesthetized and received intratracheal instillation of hCCL24. Lungs from recipient mice were collected at different time-points after the transfer and lung single-cell suspensions were prepared. Specific recruitment *in vivo* was determined by flow cytometric analysis of lung single-cell suspensions. Recruited bmEos were identified as Siglec F+/CD11c<sup>-</sup> cells in whole lung tissue. Additionally, cytopspins were prepared from lung single-cell suspensions and stained with Diff-Quik.

**Results:** Intratracheal instillation of recombinant human eotaxin-2 (hCCL24) prior to introduction of bmEos via tail vein injection results in a ~4-fold increase in Siglec F+/CD11c<sup>-</sup> eosinophils in the lungs of eosinophil-deficient *ΔdblGATA* recipient mice compared to controls ( $p > 0.05$ ). BmEos generated from CCR3-gene-deleted mice did not migrate to the lung in response to hCCL24 in this model, documenting specific receptor dependence. BmEos generated from GFP+ Balb/c mice respond to similarly hCCL24 *in vitro* and were detected in lung tissue of BALB/c wild-type as well as BALB/c *ΔdblGATA* eosinophil-deficient recipient mice, at ~4-fold (at 5 h post-instillation) and ~3-fold (at 24 h post-instillation) over baseline ( $p > 0.05$ ), respectively. Comparable results were obtained with GFP+ C57BL/6 bmEos responding to intratracheal hCCL24 in C57BL/6 *ΔdblGATA* recipient mice. Data sets were analyzed using Student's t-test.

**Conclusion:** The use of *ex vivo* cultured bmEos via one or more of these methods offers the possibility of manipulating bmEos prior to transfer into a wild-type or gene-deleted recipient host. To conclude, the development of novel *in vivo* migration models is of primary interest to researchers studying eosinophil biology and effector function with respect to respiratory diseases. This unique technique allows the manipulation of assay parameters and therefore represents a very useful tool for pharmacological studies *in vivo*.

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## POSTER 62

**MATERNAL  $\alpha$ -TOCOPHEROL SUPPLEMENTATION OF ALLERGIC FEMALE MICE INHIBITS ALLERGIC INFLAMMATION IN OFFSPRING**

Joan M. Cook-Mills, Hiam Abdala-Valencia and Sergejs Berdnikovs

Allergy/Immunology Division, Northwestern Univ. Feinberg School of Medicine, Chicago, IL

**Background:** In clinical studies, offspring of allergic mothers have increased incidence of allergic disease. It is also reported that, in mice, offspring from allergic mothers have increased risk of development of eosinophilic lung inflammation in response to suboptimal allergen challenge and it is reported that this is mediated by epigenetic changes in dendritic cells. Dendritic cell development and responses are dependent on protein kinase Ca (PKCa). We have recently demonstrated that, PKCa is directly regulated by the vitamin E isoform  $\alpha$ -tocopherol.  $\alpha$ -tocopherol binds to the C1a regulatory domain of PKCa and functions as an antagonist. It is not known whether  $\alpha$ -tocopherol regulates dendritic cell-dependent increased risk for allergic responses in offspring from allergic mothers.

**Methods:** We determined whether supplementation of allergic pregnant mice with  $\alpha$ -tocopherol blocks the development of allergic responses in the lungs of offspring. Female mice were sensitized and challenge with OVA or saline before pregnancy.  $\alpha$ -tocopherol supplemented diets were started at the time of mating. Pups were sensitized once within three days of birth and then challenged on days 7, 8, and 9 with 3% OVA. Tissues were collected on day 10.

**Results:** Pups from allergic mothers developed allergic lung responses, whereas pups from saline mothers did not respond to the OVA sensitization/challenge.  $\alpha$ -tocopherol supplementation of allergic female mice during pregnancy/lactation resulted in a dose-dependent reduction in eosinophils in the pups lungs after OVA challenge. In these pups, there was also a reduction in allergen-induced lung mRNA expression of indoleamine 2,3-dioxygenase, IL-4, IL-33, TSLP, CCL11 and CCL24.  $\alpha$ -tocopherol supplementation did not alter levels of lung leukocytes in OVA-challenged pups from saline-treated mothers.

**Conclusions:** Maternal supplementation with  $\alpha$ -tocopherol reduced development of allergic responses in offspring from allergic mothers by regulating generation of mediators of allergic inflammation. These results have implications for supplementation of allergic mothers with  $\alpha$ -tocopherol.  $\alpha$ -tocopherol supplementation of allergic mothers has the potential to reduce the incidence of allergic disease in future generations. In addition, since we have previously reported that the vitamin E isoform g-tocopherol is pro-inflammatory for allergic disease in adult mice and that g-tocopherol can oppose the anti-inflammatory benefit of  $\alpha$ -tocopherol, information about the clinical impact whereby tocopherol isoforms differentially modulate inflammation will be important in designing interventions to reduce development of asthma/allergies and control prevalence of these diseases worldwide.

**Grant support:** NIH R01 AT004837

## POSTER 63

**OZONE INDUCED AIRWAY HYPERREACTIVITY IS EXACERBATED BY BLOCKADE OF EOSINOPHIL RECRUITMENT INTO LUNGS**

Sarah A. Wicher, David B. Jacoby, Allison D. Fryer

Division of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR 97239

**Background:** Dominant autonomic control of airway smooth muscle is provided by parasympathetic nerves of the vagus. Electrical stimulation of parasympathetic nerves contracts airway smooth muscle causing bronchoconstriction. We have shown that ozone causes airway hyperreactivity that lasts at least three days and increases recruitment of inflammatory cells, including eosinophils, to guinea pig lungs. We have shown that one day after ozone, airway hyperreactivity is mediated by eosinophils (AJP. 287:627). Eosinophils enhance neurotransmitter release, resulting in increased bronchoconstriction and airway hyperreactivity. In contrast, three days after ozone, depleting eosinophils with antibody to IL-5 makes airway hyperreactivity worse, suggesting that eosinophils have acquired a protective role in chronic ozone induced airway hyperreactivity. Nerve growth factor and tumor necrosis factor are both increased in asthma, and these mediators increase adhesion molecule expression and cause airway hyperreactivity. Here we tested whether blocking either cytokine affected ozone-induced eosinophil production in bone marrow and eosinophil recruitment to the lungs.

**Methods:** Guinea pigs were treated with 100 mg/kg 5-bromo-2'-deoxyuridine (BrdU) i.p. twice daily to label newly divided eosinophils and were exposed to air or 2.0 ppm ozone for four hours. Some animals were pre-treated with 3 mg/kg etanercept i.p. twenty-four hours prior to ozone and daily thereafter, or with 10 ug/kg antibody to nerve growth factor i.p. one hour before ozone and daily thereafter. Three days after ozone exposure guinea pigs were anesthetized with 1.9 g/kg urethane i.p., paralyzed with succinylcholine i.v., and ventilated at constant volume and constant flow. Both vagi were cut and placed on platinum electrodes connected to a stimulator. Bronchoconstriction was measured as an increase in pulmonary inflation pressure (mmH<sub>2</sub>O) in response to electrical stimulation of the vagus nerves at increasing frequency. Animals were euthanized and inflammatory cells were harvested from bone marrow, blood, and lungs.

**Results:** Three days after ozone, production of newly divided eosinophils in bone marrow was increased, while other inflammatory cells were unchanged. Ozone decreased both newly divided (labeled with BrdU) and non-BrdU labeled eosinophils in blood. However, in lungs, total eosinophils were doubled and percent of newly divided eosinophils was increased from 40% to 71% three days after ozone. Blocking nerve growth factor and tumor necrosis factor did not affect bone marrow eosinophils. However, both antagonists prevented loss of new eosinophils from blood and inhibited recruitment of new eosinophils into lungs. In addition, blocking migration of new eosinophils into the lungs exacerbated ozone induced airway hyperreactivity.

**Conclusion:** Tumor necrosis factor and nerve growth factor, while not mediating bone marrow production of new eosinophils, are responsible for migration of newly formed eosinophils from the blood into the airways. However, these data suggest a novel, protective role for these cytokines and support a protective role for new eosinophils in lungs three days after exposure to ozone.

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## POSTER 64

## MOUSE MODELS TO STUDY MECHANISMS OF EOSINOPHIL SECRETION DURING INFLAMMATORY RESPONSES

Kennedy Bonjour<sup>1</sup>, Marco A. Martins<sup>2</sup>, Sandra A.C. Perez<sup>2</sup>, Haibin Wang<sup>3</sup>, Revital Shamri<sup>3</sup>, Peter F. Weller<sup>3</sup> and Rossana C.N. Melo<sup>1,3</sup>

<sup>1</sup> Laboratory of Cellular Biology, Department of Biology, Federal University of Juiz de Fora, MG, Brazil. <sup>2</sup> Laboratory of Inflammation, Oswaldo Cruz Institute. <sup>3</sup> Division of Allergy and inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School Boston, Massachusetts, USA.

**Background:** Mechanisms governing secretion of proteins underlie the functions of human eosinophils, leukocytes involved in allergic, inflammatory and immunoregulatory responses. In response to varied stimuli, eosinophils are recruited from the circulation into inflammatory foci, where they modulate immune responses through the release of granule-derived products. Eosinophils secrete proteins from their specific cytoplasmic granules through different secretion pathways: i) classical granule exocytosis; ii) piecemeal degranulation (PMD), a secretory process mediated by transport vesicles; and iii) cytolysis. These mechanisms are well characterized in humans, but are still poorly understood in mice. Here we investigated the mechanisms underlying eosinophil degranulation of murine models during inflammatory responses *in vivo* (asthma model) and *in vitro* (after stimulation with pro-inflammatory stimuli).

**Methods:** Balb/c mice were immunized with a mixed suspension of ovalbumin (OVA) (50 µg) and Al(OH)<sub>3</sub> (5 mg) in 200 µL of 0.9% NaCl on days 0 (subcutaneously) and 14 (intraperitoneally). Fourteen days later, repetitive OVA challenges (50 µg) were intranasally instilled 3 times a week during 4 weeks. The control group received 0.9% NaCl solution. After treatment, lung fragments were fixed and processed for light and transmission electron microscopy (TEM). Moreover, interleukin-13 (IL-13), IL-4, eotaxin-1 (CCL11) and transforming growth factor beta (TGF-β) were measured in the lung tissue. For the *in vitro* experiments, eosinophils were isolated from the spleen of interleukin-5 transgenic mice, stimulated with GM-CSF (10 ng/mL), LPS (100 ng/mL) or medium alone for 1h, immediately fixed and processed to TEM.

**Results:** Both light and TEM revealed an intense eosinophil infiltration in perivascular and peribronchial spaces of OVA-treated animals, but these cells were rarely observed in control lungs. Ultrastructural analysis of 456 secretory granules from infiltrating eosinophils showed that 36% of these granules had degranulation signs associated with PMD, characterized mainly by reduced electron density, losses in the matrix or cores, coarse matrix and/or granule enlargement. Vesicles were visualized nearby emptying granules. Eotaxin-1, IL-4, IL-13 and EPO levels in the lung tissue from OVA-stimulated mice were significantly increased compared to controls while the levels of TGF-β did not change. Spleen eosinophils stimulated *in vitro* with GM-CSF and LPS exhibited shape changes and around 20% of emptying granules. Granule-granule or plasma membrane-granule fusions were not observed in any situation. Interestingly, we identified by TEM a distinct electron-dense brim at specific areas of the limiting granule membrane, a novel morphological feature which may be associated with secretion in mice eosinophils.

**Conclusions:** Collectively, our data demonstrate that mouse eosinophils are able to degranulate through PMD during inflammatory responses occurring both *in vivo* and *in vitro*. Although the morphological features recognized as indicative of PMD are less pronounced in mice compared to those documented in humans, these signs can be detected in mice eosinophils under careful investigation by TEM. Moreover, our study demonstrate that other morphological signs, not described for human eosinophils, may be helpful to understand the secretory processes in eosinophils from mice models.

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## POSTER 65

**DIRECTED DELIVERY OF ANTIGEN TO RESPIRATORY MUCOSAL SURFACES; A MOUSE MODEL TO ADDRESS LOCAL AND REMOTE MUCOSAL EFFECTS IN ALLERGIC AIRWAY INFLAMMATION**

Maytal Bivas-Benita, Jason J. Xenakis, Samuel D. Maldonado, Linying Liu, and Lisa A. Spencer

Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115 USA

**Background:** Current mouse models of allergic airway inflammation deliver allergen challenges to respiratory mucosa intranasally or by inhaled aerosols. These methodologies lack precision in allergen dosing. Moreover, a portion of allergen is inevitably swallowed, resulting in concomitant exposure of intestinal mucosa, and preventing investigations of local versus remote aspects of mucosal allergen exposure.

We sought to 1) adapt a method of non-invasive microspray administration of allergen directly to lung surfaces, and 2) utilize this method to investigate the impact of respiratory allergen exposure on mouse intestinal eosinophils.

**Methods:** Pulmonary allergen exposures were performed using a non-invasive lung aerosolization technique in anesthetized mice. To assess aerosol distribution, 50µl of a 0.6% India Ink solution in PBS were sprayed directly into lungs using the MicroSprayer® Aerosolizer (Penn-Century, Inc. Wyndmoor, PA). Mice were sacrificed 20 minutes later and lungs evaluated for ink distribution. In allergen sensitization studies, female BALB/c mice were sensitized intraperitoneally with ovalbumin (OVA) in an aluminum hydroxide (alum) containing solution on days 0, 7 and 14. Control animals received PBS in alum. Seven days after final sensitization, mice received 20µg of OVA in 50µl PBS, or 50µl PBS alone, sprayed directly into lungs on three consecutive days. Mice sacrificed four days after final lung challenge were assessed for airway inflammation, systemic eosinophilia, and intestinal eosinophilic infiltration using traditional histological (H&E, PAS, and fast green/neutral red staining of paraffin sections) and flow cytometric (eosinophils identified as SSC<sup>hi</sup>SiglecF<sup>+</sup>CCR3<sup>+</sup>) analyses.

**Results:** Dye delivered by microspray was deposited prominently in all areas of left lung, with staining also evident in right lung bronchi, but not right lung alveoli. In some mice, sprayed dye was also observed in the lower portion of the trachea. Similar to conventional allergen challenge methods, OVA microsprayed into lungs of sensitized (but not naïve) mice elicited classic signs of eosinophilic tissue inflammation, eosinophil accumulation within bronchoalveolar space (>30% total BAL leukocytes), and systemic eosinophilia, evidenced by increased numbers of eosinophils within blood and bone marrow. Moreover, remote respiratory exposure to OVA in sensitized mice elicited increases in the number of intestinal lamina propria eosinophils, both in regions surrounding crypts and extending throughout villi. Histological counts were supported by flow cytometry of isolated intestinal lamina propria leukocytes, whereby eosinophil percentages increased in OVA/alum sensitized and lung OVA challenged mice in comparison to mice sensitized with PBS/alum and challenged with PBS.

**Conclusions:** Direct administration of allergen to mouse lungs using the MicroSprayer® Aerosolizer enabled delivery of allergen that could be accurately quantified and spatially targeted within airways. OVA microsprayed into lungs of sensitized, but not naïve, mice elicited classic signs of allergic airway inflammation, and increased numbers of intestinal eosinophils. Therefore, our findings reveal cross-talk between remote mucosal systems impacting intestinal eosinophil populations.

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## POSTER 66

**PREVENTION OF INFLAMMATORY COLITIS BY A RECOMBINANT ENZYME FROM THE SCHISTOSOME HELMINTH PARASITE: INDUCTION OF A TH2 RESPONSE WITH EOSINOPHILS AND ALTERNATIVELY ACTIVATED MACROPHAGES**

Capron M.<sup>1</sup>, El Nady M.<sup>1\*</sup>, Delbèke M.<sup>1</sup>, Driss V.<sup>1</sup>, Dubuquoy L.<sup>1</sup>, Rousseaux C.<sup>2</sup>, Dubuquoy C.<sup>2</sup>, Gatault S.<sup>1</sup>, Colombel J.F.<sup>1,3</sup> & Desreumaux P.<sup>1</sup>.

<sup>1</sup>U 995 Inserm University Lille<sup>2</sup> Medical School, Lille, France

<sup>2</sup> Intestinal Biotech Development, Lille, France

<sup>3</sup> Icahn School of Medicine at Mount Sinai, New York, USA

\*Present address: Department of Internal Medicine, Cairo University, Egypt

Helminth parasites are potent immunoregulators of inflammatory diseases. Although infection with schistosomes reduces intestinal inflammation, the molecules responsible for this anti-inflammatory effect are still unknown.

**Methods and Results:** Immunization with a recombinant Glutathion *S*-transferase from schistosomes, the P28GST, known to induce a Th2 response, prevented experimental colitis induced by the TNBS hapten in Sprague Dawley rats and in C57Bl/6 or BALB/c mice. Clinical and histological scores of colitis were significantly reduced (up to 50%), in immunized animals, by comparison with controls. Up to 90% decrease in myeloperoxidase evaluated by Elisa and 50% decrease in mRNA expression of pro-inflammatory cytokines (IL-1 $\beta$ , IL-17 and TNF) were observed in colonic tissues of immunized animals. This potent anti-inflammatory effect was associated with a shift in the immune response characterized by a decrease in Th1- associated IFN $\gamma$  and increased expression of Th2 -associated IL-4, IL-5 and IL-13. Immunohistochemistry analysis of colonic sections revealed a major eosinophil infiltration and Arginase positive cells in immunized rats, as well as an increase in the ratio of mRNA expression of Arg1/iNOS2. These results indicated the presence of alternatively activated macrophages (AAMs), characterized by their anti-inflammatory effect and association with a Th2 immune response. Thus, immunization with the schistosome P28GST promoted local eosinophils and AAMs implicated in the control of colitis

**In conclusion:** This study provides the first evidence that immunization with a recombinant protein from the Schistosome helminth parasite prevents hapten-induced colitis in two models of rodents. Studies looking at the efficacy and safety of P28GST in the treatment of inflammatory bowel disease in humans are in progress. .

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## POSTER 67

## THE CLINICAL PROFILE OF UK ASTHMA PATIENTS WITH RAISED BLOOD EOSINOPHILS

Anna Rigazio<sup>1</sup>, David B. Price<sup>2</sup>, Jonathan D. Campbell<sup>3</sup>, Christopher J. Corrigan<sup>4</sup>, Ian D. Pavord<sup>5</sup>, Sally E. Wenzel<sup>6</sup>, Francesca Barion<sup>1</sup>, Shuna Gould<sup>1</sup>, Julie von Ziegenweidt<sup>1</sup>, Philip O. Buck<sup>7</sup>, Gokul H. Gopalan<sup>7</sup>, Mary Buatti Small<sup>7</sup>.

(<sup>1</sup>) Research in Real Life, Cambridge, UK, (<sup>2</sup>)University of Aberdeen, UK, (<sup>3</sup>) University of Colorado, CO, (<sup>4</sup>) King's College, London, UK, (<sup>5</sup>) Glenfield Hospital, Leicester, UK, (<sup>6</sup>) University of Pittsburgh, PA, (<sup>7</sup>) Teva Pharmaceuticals, Frazer, PA.

**Background:** One of the hallmarks of asthma is increased numbers of eosinophils in the airway mucosa. A proportion of patients also have blood eosinophilia. However, how this relates to clinical endpoints remains unclear. We set out to explore the relationship between blood eosinophil counts, asthma exacerbations and patient asthma control using a large primary care-based database.

**Methods:** Patient data were obtained from the Clinical Practice Research Database, the English NHS longitudinal research database, and the Optimum Patient Care Research Database, which collect primary care data in the UK supplemented with information from patient-reported questionnaires. Patients included in the study were aged 12 – 80 years with a diagnostic code for asthma and recorded blood eosinophil count. Patients with diagnostic codes for any other chronic respiratory disease, or using only reliever therapy for asthma, were excluded. Blood eosinophil counts were the last values recorded before the data extraction and were categorized as either  $\leq 400$  or  $>400 \times 10^6/L$ . The cut-off  $400 \times 10^6/L$  represents the upper limit of the normal range in the UK. Outcomes were exacerbations (asthma-related hospital admission or Emergency Room attendance or use of acute oral steroids) and asthma control (defined as absence of exacerbations and GP consultations for lower respiratory tract infections associated also with an average daily dose  $\leq 200$ mcg of salbutamol) during the year prior to data extraction.

**Results:** From the 448,859 asthma patients aged 12-80 with no other chronic respiratory disease, 60.6% had a recorded eosinophil count. 102,802 of these were excluded as they only received short-acting  $\beta$ -agonist treatment. 83% of 110,949 patients fulfilling all inclusion criteria had blood eosinophil counts of  $\leq 400 \times 10^6/L$ . The proportion of patients with controlled asthma was 38.6% in the group with low counts and 35.8% in the group with high counts ( $p < 0.001$  Chi squared test). One, 2-3 and 4+ exacerbations occurred in 13.2%, 5.4% and 2% of patients with low counts and 14.2%, 6.9% and 2.9% of patients with high counts ( $p < 0.001$ , Chi squared test).

The relationship between blood eosinophil count and the proportion of patients with controlled disease was approximately linear ( $r=0.169$ ;  $p=0.006$ ; figure). For a  $1000 \times 10^6/L$  increase in eosinophil counts, the proportion of patients with controlled asthma is expected to decrease by 1.79% (95% CI=-3.06,-0.52; figure).

**Conclusions:** The data suggest that patients with blood eosinophils counts in excess of  $400 \times 10^6/L$  tend to suffer from more exacerbations and poorer asthma control and the higher the eosinophil count, the less likely it is they achieve asthma control.

*Study funded by TEVA*

## POSTER 68

## ASPIRIN-INDUCED ASTHMA SUB-PHENOTYPES IDENTIFIED BY LATENT CLASS ANALYSIS

Grazyna Bochenek<sup>1</sup>, Joanna Kuschill-Dziurda<sup>1</sup>, Krystyna Szafranec<sup>2</sup>, Hanna Plutecka<sup>1</sup>, Ewa Nizankowska-Mogilnicka<sup>1</sup>

<sup>1</sup>Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland

<sup>2</sup>Department of Epidemiology and Population Studies, Faculty of Health Sciences, Jagiellonian University Medical College, Krakow, Poland

**Background:** Aspirin-induced asthma (AIA), known also as aspirin-exacerbated respiratory disease (AERD), is a distinct asthma phenotype with frequent coexistence of chronic hyperplastic eosinophilic rhinosinusitis with nasal polyps whose symptoms are exacerbated by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) – nonselective inhibitors of cyclooxygenase 1. It is generally recognized as a severe type of asthma accompanied by increased blood eosinophil count. Most AIA patients synthesize excessive amounts of cysteinyl leukotrienes in a stable condition, which is reflected by an increased urinary leukotriene E4 (LTE4) concentrations when compared with the asthma patients who tolerate aspirin well. More insightful analysis of this group reveals that it is not quite homogenous, as individual patients differ in terms of specific clinical and laboratory parameters. The present study aimed to identify the likely AIA sub-phenotypes by applying advanced statistical modelling methods.

**Methods:** We enrolled to the study 201 AIA patients (134 females, 67 males, mean age 49.4 yrs) in whom the diagnosis was confirmed by typical history and a positive aspirin challenge. Clinical data were collected using specifically structured questionnaire. The levels of asthma severity and control were assessed according to NAEPP EPR3 guideline. Standard spirometry, skin prick tests, blood eosinophil count, urinary LTE4 concentrations were evaluated. In order to identify possible AIA sub-phenotypes, latent class analysis method was applied. This method groups together patients according to the similarities of the specific clinical and laboratory features.

**Results:** By applying a novel biostatistical modelling approach it was possible for the first time to distinguish four sub-phenotypes (latent classes) within the AIA cohort. Class 1 comprised 18.9% of patients with a moderate course of asthma, intensive upper airways symptoms and increased blood eosinophil count. Class 2 comprised 34.8% of patients with a mild course of asthma, relatively well controlled, with low health care use. Class 3 embraced 41.3% of patients with a severe course of asthma, which was poorly controlled, with severe exacerbations and airway obstruction. Class 4 embraced 5.0% of patients, only females with poorly controlled asthma and frequent severe exacerbations. Even though all classes comprised a high proportion of patients with upper airways symptoms, Class 1 was distinguishable in this regard. Moreover, Class 1 covered the highest proportion of patients with increased blood eosinophil count and the highest concentration of LTE4 in urine. Particularly intensive upper airways disease in this class was not necessarily interrelated with a severe course of asthma.

**Conclusions:** Latent class analysis revealed that AIA patients do not constitute quite homogenous population. Patients with the most intensive upper airways symptoms had the highest blood eosinophil count and the highest concentrations of urinary LTE4, which could be directly related to an abundant chronic eosinophilic inflammation of their upper airways.

## POSTER 69

**IDENTIFICATION OF GENES EXPRESSED BY HUMAN AIRWAY EOSINOPHILS AFTER AN *IN VIVO* ALLERGEN CHALLENGE**

Stephane Esnault<sup>1</sup>, Elizabeth A. Kelly<sup>1</sup>, Elizabeth A. Schwantes<sup>1</sup>, Lin Ying Liu<sup>1</sup>, Larissa P. DeLain<sup>1</sup>, Jami A. Hauer<sup>1</sup>, Yury A. Bochkov<sup>2</sup>, Loren C. Denlinger<sup>1</sup>, James S. Malter<sup>3</sup>, Sameer K. Mathur<sup>1</sup>, Nizar N. Jarjour<sup>1</sup>

<sup>1</sup>Department of Medicine, Allergy, Pulmonary, and Critical Care Medicine Division, <sup>2</sup>Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI USA, <sup>3</sup>Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX USA

**Background:** Eosinophilic inflammation is a hallmark of allergy in general and particularly allergic asthma. However, the mechanism for their contribution to asthma pathophysiology is not fully understood. Airway allergen challenge triggers eosinophil (EOS) differentiation and airway recruitment, and thus is a powerful and physiologically relevant human model to understand the biology and the function of EOS in allergy and asthma. Genome-wide expression analysis of airway EOS by microarrays has been limited by the ability to generate high quality RNA from sufficient numbers of airway EOS.

**Objective:** To identify, by genome-wide expression analyses, a compendium of expressed genes characteristic of sputum and airway EOS following allergen challenge.

**Methods:** Atopic, mild asthmatic subjects were recruited for these studies. Induced sputum was obtained before and 48h after a whole lung allergen challenge (WLAC). Individuals also received a segmental bronchoprovocation with allergen (SBP-Ag) 1 month before and after administering a single dose of mepolizumab (anti-IL-5 monoclonal antibody) to reduce airway EOS. Bronchoalveolar lavage (BAL) was performed before and 48 h after SBP-Ag. Gene expression of sputum and BAL cells was analyzed by microarrays. The results were statistically validated by qPCR in BAL cells and purified BAL EOS from 6 subjects.

**Results:** A total of 299 transcripts were up-regulated by more than 2-fold in total BAL cells following SBP-Ag. Mepolizumab treatment resulted in a reduction of airway eosinophils by 54.5% and decreased expression of 99 of the 299 transcripts. 3 of 6 post-WLAC sputum samples showed increased expression of EOS-specific genes (RNASE2, RNASE3, IL5RA and SIGLEC8), along with the expression of 361 other genes. Finally, the intersection of the 3 groups of transcripts (increased in BAL post SBP-Ag (299), decreased after mepolizumab (99), and increased in sputum after WLAC (365)) was composed of 57 genes characterizing airway EOS gene expression.

**Conclusion:** We identified 57 genes highly expressed by airway EOS compared to unseparated BAL cells after *in vivo* allergen challenge. 41 of these genes had not been previously described in EOS and are thus potential new candidates to elucidate EOS contribution to airway pathophysiology in asthma and possibly other diseases.

*This work was supported in part by a Program Project Grant (NIH HL088594) and the University of Wisconsin Institute for Clinical and Translational Research (NCRR/NIH 1UL1RR025011). Mepolizumab was provided by GlaxoSmithKline.*



## POSTER 70

**SEMAPHORIN 7A IS EXPRESSED ON HUMAN AIRWAY EOSINOPHILS AFTER SEGMENTAL ALLERGEN CHALLENGE *IN VIVO* AND IS UPREGULATED BY THE COMMON  $\beta$  CHAIN CYTOKINES *IN VITRO***

Stephane Esnault<sup>1</sup>, Elizabeth A. Kelly<sup>1</sup>, Mats W. Johansson<sup>2</sup>, Shih-Tsung Han<sup>2</sup>, Moneeb Akhtar<sup>1</sup>, Nate Sandbo<sup>1</sup>, Dean F. Mosher<sup>2</sup>, James S. Malter<sup>3</sup>, Loren C. Denlinger<sup>1</sup>, Sameer K. Mathur<sup>1</sup>, Nizar N. Jarjour<sup>1</sup>

<sup>1</sup>Department of Medicine, Allergy, Pulmonary, and Critical Care Medicine Division, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA <sup>2</sup>Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

<sup>3</sup>Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

**Background:** Semaphorin 7A plays a major role in TGF- $\beta$ 1-induced lung fibrosis and it increases pro-inflammatory cytokine release by antigen presenting cells. Semaphorin 7A is known to be expressed on T cells, macrophages, and airway epithelial cells; however, its presence on eosinophils has not been explored.

**Objective:** Based on the accumulating evidence that eosinophils contribute to immune regulation and fibrosis/remodeling in the airway, we hypothesized that airway eosinophils may be a significant source of semaphorin 7A.

**Methods:** *In vivo*, expression of semaphorin 7A on human eosinophils obtained from the circulation and bronchoalveolar lavage after a bronchoscopic segmental bronchoprovocation with allergen was analyzed by real-time PCR and flow cytometry. Semaphorin 7A regulation on eosinophils by the common  $\beta$  chain family cytokines (IL-5, GM-CSF and IL-3) was studied *in vitro*.

**Results:** Semaphorin 7A was expressed on circulating eosinophils and was upregulated on bronchoalveolar lavage eosinophils. Based on mRNA levels in bronchoalveolar cells and purified airway eosinophils, eosinophils appeared to be a predominant source of semaphorin 7A. *In vitro*, semaphorin 7A protein of blood eosinophils were increased by the common  $\beta$  chain cytokines with a more potent effect from IL-3 compared to GM-CSF or IL-5. In addition, IL-3-activated blood eosinophils were highly adherent to the semaphorin 7A-specific ligand plexin C1.

**Conclusion:** Our findings demonstrate high expression of semaphorin 7A on airway eosinophils and its regulation by the common  $\beta$  chain-signaling cytokines, principally IL-3. Semaphorin 7A might be another tool used by eosinophils to increase both lung fibrosis and the expression of pro-inflammatory cytokines by monocytes/macrophages.

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## POSTER 71

**WHOLE BODY COUNTING TO QUANTIFY THE DISTRIBUTION AND FATE OF INDIUM-111-LABELLED EOSINOPHILS IN HEALTHY AND ASTHMATIC VOLUNTEERS**

Neda Farahi<sup>1</sup>, Chrystalla Loutsios<sup>1</sup>, Daniel Gillett<sup>2</sup>, Sarah Heard<sup>2</sup>, Rosalind Simmonds<sup>1</sup>, A. Mike Peters<sup>3</sup>, Alison M. Condliffe<sup>1</sup> and Edwin R. Chilvers<sup>1</sup>

<sup>1</sup>Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, UK; <sup>2</sup>Nuclear Medicine, Addenbrooke's Hospital, CUHNHSFT, Cambridge, UK; <sup>3</sup>Clinical Sciences Imaging Centre, Brighton and Sussex Medical School, Brighton, UK.

**Background:** Eosinophils are key mediators of allergic inflammation in asthma. The Whole Body gamma Counter (WBC) provides a way to study the whole body distribution and loss of radiolabelled autologous granulocytes using very low quantities of radioactivity. This study was designed to determine whether there is a difference in the whole body retention of eosinophils over time between asthmatics and healthy individuals, and to ascertain the fate and distribution of eosinophils in healthy individuals and in those with asthma.

**Methods:** Autologous Indium (In)-111-labelled eosinophils were prepared using clinical grade anti-CD16 immuno-magnetic beads as described previously (1). Eosinophils were labelled with In-111-tropolonate (administered activities ranged from 0.2 to 2.37MBq) and re-injected into 4 healthy subjects and 5 asthmatics. Radioactivity was measured from head to toe at intervals up to 7 days using a heavily-shielded WBC. Anterior and posterior detectors were fitted with slit collimators to generate longitudinal whole body profiles of In-111-labelled eosinophil distribution. In addition, expectorated sputum was collected daily and radioactivity counts measured using a gamma counter.

**Results:** In both healthy subjects and asthmatics, In-111-labelled eosinophils migrated mainly to the liver and spleen. In contrast to neutrophils, these cells exhibited markedly less redistribution to the bone marrow after 24 hours. There was no difference in In-111 eosinophil loss between the healthy ( $11 \pm 1.7\%$ ) and asthmatic volunteers ( $14.4 \pm 3.6\%$ ) over 7 days. In two of the asthmatic subjects, enhanced radioactivity was observed over the thoracic region. Sputum from asthmatics was radioactive with peak counts demonstrated in the first 24-hours following re-injection.

**Conclusions:** These results demonstrate for the first time that radiolabelled eosinophils injected into the blood of asthmatics migrate to the lungs and enter the lumen of the airway before being expectorated in the sputum. They also highlight the differences in the physiological distribution and fate of eosinophils compared to neutrophils. This further enhances our knowledge of the behaviour of eosinophils in health and asthma.

(1) Farahi et al. Use of 111-Indium-labeled autologous eosinophils to establish the *in vivo* kinetics of human eosinophils in healthy subjects. *Blood* 2012; 120(19):4068-4071

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**POSTER 72****EOSINOPHILS AND NASAL ALLERGEN CHALLENGE**

Leon Carayannopoulos<sup>1\*</sup>, Vlad Malkov<sup>1\*</sup>, Marcella K. Ruddy<sup>1</sup>, George Tokiwa<sup>1</sup>, Robin Mogg<sup>1</sup>, Hyun Chung<sup>1</sup>, Inge DeLepeleire<sup>1</sup>, Guang Chen<sup>1</sup>, Brian R. Leaker<sup>2</sup>, Trevor T. Hansel<sup>3</sup>

<sup>1</sup>Merck Research Laboratories, Merck Sharp & Dohme; <sup>2</sup>Respiratory Clinical Trials (RCT), London; <sup>3</sup>Imperial Clinical Respiratory Research Unit (ICRRU) at St Mary's Hospital, Imperial College, London.

**Background:** Nasal allergen challenge (NAC) is a minimally invasive human model with which to study upper airway mucosal responses to allergen. Following NAC we performed serial non-invasive sampling without anaesthesia by nasosorption, nasal lavage and nasal epithelial curettage.

**Methods:** A randomized, double-blind, placebo-controlled, 3-period cross-over study was performed in 19 patients with allergic rhinitis out of season. Single-dose oral prednisone (10mg or 25mg) or placebo was administered 1h before challenge with grass pollen extract administered by nasal spray. Serial sampling of nasal mucosal lining fluid (MLF) was performed using strips of synthetic absorptive matrix (SAM) by the technique of nasosorption, eluted samples being analysed for levels of cytokines and chemokines by multiplex immunoassay. Epithelial curettage samples (Rhinoprobe) were taken at baseline and 6h post NAC, and whole genome expression performed using microarray. We also used gene-gene expression correlation data as well as immune cell selective gene expression databases to identify cell type-associated genes in nasal scrapes.

**Results:** After NAC approximately half of subjects produce IL-5 and IL-13 at a protein and gene expression level, levels becoming detectable in MLF at 2 to 8h post NAC. This cytokine response is correlated with the influx of eosinophils measured by gene expression in mucosal curettage samples. Among granulocyte cell-specific mRNA markers: carbohydrate-binding proteins were prominent for eosinophils (CLC, SIGLEC8, EMR1, EMR4P, LGALS12), neutrophils have certain cell surface receptors (FCGR2A, FCGR2C, CXCR1, CXCR2, FPR1), while mast cells have intracellular proteases (TPSAB1, TPSB2, CPA3).

A single dose of corticosteroid was found to generally inhibit IL-5, IL-13 and eosinophil numbers, with evidence of a dose response, but this effect varied between different subjects. There was a lesser effect of NAC in causing influx of neutrophils, and this was also inhibited by corticosteroid. In contrast, genes associated with plasmacytoid dendritic cells are reduced after NAC, but this decrease is not prevented by prednisone.

**Conclusions:** The nasal mucosal allergic reaction is variable between individuals in terms of the Th2 pathway and response to corticosteroid. This NAC approach may help stratification of atopic asthmatics into potential responder patients for specific Th2-directed therapy.

**Funding:** *This study was funded by Merck Research Laboratories, Merck Sharp & Dohme.*

**POSTER 73****ROLE OF CCL26 IN THE RECRUITMENT OF HUMAN EOSINOPHILS: A PUTATIVE ROLE IN ASTHMA SEVERITY**

Nicolas Flamand, Marie-Chantal Larose, Jamila Chakir, Christian Couture, Véronique Provost, Michel Laviolette.

Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Faculté de médecine, Université Laval, Québec, QC, Canada,

**Background:** Eotaxins can activate CCR3 and its blockade prevents the development of experimental asthma in rodents. However, it only impairs the recruitment of eosinophils by ~40% in humans. In addition to CCL11 and CCL24, humans also express CCL26 and we postulated that this eotaxin might be expressed during asthma and participate to eosinophil migration in this disease. We therefore assessed the impact of CCL26 on human eosinophils migration and the cellular mechanisms involved. We also evaluated the expression of CCL26 by IL-13-stimulated bronchial epithelial cells obtained from asthmatics of different severities and bronchial biopsies.

**Methods:** Eosinophil migration through a reconstituted basement membrane was evaluated using Matrigel™ invasion chambers for up to 18 hours in presence of CCL11, CCL24 and CCL26 as chemotactic factors. Bronchial biopsies were obtained from healthy controls, mild and severe eosinophilic asthmatics and bronchial epithelial cells lines were established from these tissues. CC chemokine expression was measured by qPCR from IL-13-stimulated bronchial epithelial cells and eotaxin expression was analysed by immunohistochemistry on bronchial biopsies.

**Results:** The migration of eosinophils of healthy volunteers was similar for the three eotaxins. However, eosinophils of mild asthmatics had a greater response to CCL11 and a much greater response to CCL26 compared to CCL24. CCR3 blockade completely abrogated the effect of the three eotaxins on eosinophils of healthy subjects. Interestingly, CCR3 blockade did not affect the migration induced by CCL26 on eosinophils of asthmatics. IL-13-stimulated bronchial epithelial cells predominantly increased CCL26 mRNA compared to all the other human chemokines. Moreover, this expression of CCL26 was greater and sustained in bronchial epithelial cells from severe eosinophilic asthmatics. CCL26 expression in bronchial biopsies also increases with asthma severity.

**Conclusions:** CCL26 is a more effective chemoattractant than CCL11 and CCL24 for eosinophils of asthmatics. The mechanism of this greater efficiency is not yet defined but our data support the hypothesis that CCR3 is not the only receptor mediating the biological effects of CCL26 and that additional receptor(s) are expressed on eosinophils of asthmatics. Moreover, CCL26 is the predominant CC chemokines expressed by BEC, and this expression increased in severe eosinophilic asthma. These results suggest that CCL26 may play a unique and important role in the recruitment of eosinophils in persistent asthma.

**Grant support:** *the Canadian Institutes of Health Research and J-D-Bégin Pulmonary Chair Research Grant.*

**POSTER 74****DURING INFLAMMATION REGRATORY EOSINOPHILS STAY IN PERIPHERAL BLOOD WHILE ACTIVATED COUNTERPARTS DWELL IN TISSUE**Bart Hilvering

**Background:** The dynamics of eosinophil and neutrophil homing to, and activation in, the tissue plays an important role in chronic inflammatory lung diseases such as asthma. Sputum analysis is very important to study this dynamics, and can also be used to distinguish between eosinophilic and non-eosinophilic asthma, but is a variable and invasive procedure. We designed a clinical cohort study to compare eosinophils in sputum and blood of asthma patients (AIR-study, NCT01611012) to study the correlation between blood and sputum granulocytes and to possibly facilitate the diagnosis of eosinophilic and non-eosinophilic asthma

**Objective:** To assess whether the expression of active FcγRIIa (CD32) and MAC-1 (CD11b/CD18) on blood eosinophils in presence and absence of the innate immune stimulus fMLF predicts the presence of eosinophils in induced sputum.

To assess the diagnostic value of non-invasive measurements: eosinophil characteristics in peripheral blood and FeNO for the diagnosis of eosinophilic versus non-eosinophilic asthma compared to routine diagnosis based on sputum phenotypes.

**Methods:** 70 asthma patients were recruited at the outpatient clinic of the UMCU. Eosinophils in whole blood were stained with a FITC labeled antibody directed against active FcγRIIa receptor (clone A17) and a PE-labeled antibody against the alpha-chain of the Mac-1 (CD11b) complex in the absence and presence of the activator fMLF (1 μM). Subsequently, the expression of activation markers was measured by flow cytometry, and data were compared with similarly processed sputum cells. Patient's characteristics and FeNO were obtained at the same time point.

**Results:** When eosinophils were present in sputum (22 patients with >0,1% eosinophils in sputum versus 38 without), blood eosinophils express significantly less CD11b ( $p=0,04$ , Mann-Whitney U-test) and active CD32 ( $p=0,04$  Mann-Whitney U-test) after stimulation with fMLF. The second finding was that the percentage of eosinophils in sputum correlates strongly with the Fraction of exhaled Nitric Oxide (FeNO in ppb.).

**Conclusion:** Differences in eosinophil activation characteristics in peripheral blood correlate with the presence of eosinophils in sputum. It is tempting to speculate that 'activation prone' eosinophils migrated to the lung, while 'refractory cells' stayed in peripheral blood. This non-invasive blood measurement, in combination with FeNO can distinguish between eosinophilic and non-eosinophilic asthma and might ultimately replace sputum induction as non-invasive test to diagnose the inflammatory phenotype of asthma.

**POSTER 75****PEG-INTERFERON TREATMENT OF EOSINOPHILIC OTITIS MEDIA-REPORT OF 2 CASES**

JH Butterfield, M.D.<sup>1</sup>, Brian Neff, M.D.<sup>2</sup>

<sup>1</sup>Division of Allergic Diseases; <sup>2</sup>Division of Otorhinolaryngology Mayo Clinic, Rochester, Minnesota, USA

**Background:** Eosinophilic otitis media (EOM) is an intractable, chronic middle ear disorder characterized by local accumulation of eosinophils and highly viscous middle ear effusions. Treatment has been difficult and no lasting cures have been achieved. Here we report successful treatment of EOM with PEG-Interferon.

**Methods:** The clinical and laboratory responses to PEG-Interferon of two patients with EOM are presented.

**Results:** The first patient was a 45 year old man with a 15 year history of difficult to control asthma, 3 prior nasal polypectomies and bilateral tympanomastoidectomies for inflammatory mastoiditis and removal of granulation tissue. The second patient was a 54 year old man with Samter's triad and a 3-year history of bilateral otitis media that began following a nasal polypectomy. He had bilateral tympanic membrane perforations and thick mucoid effusions. The peak peripheral eosinophil count for each patient was 520/mm<sup>3</sup> and 1990/mm<sup>3</sup>. The middle ear effusion level of eosinophil cationic protein (ECP) measured in patient #2 and was markedly elevated when compared to middle ear effusion ECP levels of control patients. The effusions had been responsive only to oral prednisone (pt #1) or injections of triamcinolone acetonide (pt #2) and were so tenacious that multiple sets of ear tubes had been expelled in each of the cases. Weekly PEG-Interferon was started by subcutaneous injection at a dose of 1 microgram per kilogram and resulted in normalization of the peripheral eosinophil counts. Co-incidentally with control of eosinophilia, the effusions dried up and hearing improved. One patient has continued to do well on a progressive taper of PEG-Interferon. The second patient discontinued PEG-Interferon due to side effects and experienced recurrent plugging of his tympanostomy tubes.

**Conclusions:** EOM responds to control of eosinophilia. PEG-Interferon appears to be an alternative, steroid-sparing treatment for some patients with EOM with long-term benefit.

**POSTER 76****GLEICH'S SYNDROME OF THE GUT: A NEW DISORDER OR AN UNDER-RECOGNIZED PHENOMENON?**

Paneez Khoury<sup>1</sup>, Oral Alpan<sup>2</sup>, Denise Loizou<sup>2</sup>, Jeananne Ware<sup>1</sup>, Amy D. Klion<sup>1</sup>.

<sup>1</sup> Laboratory of Parasitic Diseases, NIAID, NIH. Bethesda, MD

<sup>2</sup> Section on Immunopathogenesis, O & O ALPAN, LLC. Fairfax, VA

**Background:** Gleich's syndrome is characterized by monthly cycles of urticaria, angioedema and peripheral eosinophilia with resolution of symptoms and eosinophilia in between cycles. During a flare of Gleich's syndrome, biopsies of the skin demonstrate eosinophilic infiltration and deposition of eosinophil granule proteins. The reason that eosinophils preferentially localize to the skin and soft tissues in this disorder is unknown. A 9 year old boy with recurrent abdominal pain, nausea, and diarrhea was noted to have peripheral eosinophilia. Endoscopy demonstrated a mild increase in lamina propria eosinophils in the duodenum. Food avoidance guided by a combination of skin-prick and patch testing was attempted but did not prevent recurrent episodes.

**Methods:** After secondary causes of eosinophilia including parasitic, rheumatologic, and complement disorders were excluded, complete blood counts with differentials were performed and serum samples collected every 2-3 days over a 110 day period. Symptoms were recorded in a daily log. Repeat upper endoscopy with biopsies was performed in the absence of symptoms, and again during the onset of a flare. Serum cytokine levels were measured using suspension array technology in multiplex.

**Results:** Serial monitoring revealed episodic exacerbations of gastrointestinal symptoms temporally related to rising peripheral eosinophilia every 50-55 days. Resolution of eosinophilia occurred within 20 days of onset without specific therapy or changes in diet. Eosinophils were not demonstrated in tissue biopsies of the duodenum taken when the patient was asymptomatic. Repeat endoscopy during a flare of abdominal pain, demonstrated 64 eosinophils/hpf in the duodenum. Serum IL-5 levels demonstrated a cyclic pattern peaking prior to the peak of peripheral eosinophilia, as has been reported in Gleich's syndrome.

**Discussion:** Cyclic gastrointestinal eosinophilia may represent an unrecognized disorder with some similarities to Gleich's syndrome. The reason for eosinophilic tissue recruitment to the gastrointestinal tract rather than the skin is unknown. The timing of endoscopic procedures is critical for making the diagnosis and should be performed at the time of symptoms when tissue eosinophilia is present. Appropriate differentiation of this clinical entity from flares of eosinophilic gastrointestinal disease is important as it may alter the approach to therapy.

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## POSTER 77

**HEMATOLOGIC MALIGNANCIES IN PATIENTS WITH HYPEREOSINOPHILIA AND HYPEREOSINOPHILIC SYNDROME**

Jay J. Jin, M.D., Ph.D.<sup>1</sup>, Joseph H. Butterfield, M.D.<sup>2</sup>, Catherine R. Weiler, M.D., Ph.D.<sup>2</sup>

<sup>1</sup> Department of Internal Medicine, <sup>2</sup> Division of Allergic Diseases, Mayo Clinic, Rochester, Minnesota, USA

**Background:** Hypereosinophilia (HE) and hypereosinophilic syndromes (HES) have been associated with the development of hematologic malignancies. This study was designed to determine the overall frequency and types of hematologic malignancy that develop in hypereosinophilic states, and to determine median survival upon diagnosis of a hematologic malignancy.

**Methods:** We performed a retrospective case-series of patients diagnosed with HE or HES who subsequently developed a hematologic malignancy between the years 2000 and 2011. A chart review of the Mayo Clinic database identified adult (>18 years of age) patients with HE or HES based on diagnostic codes. This subset of charts was then reviewed only for diagnostic codes corresponding to lymphoma and leukemia. Patients lost to follow-up were not separately contacted to determine outcomes. The mean time from diagnosis of HE or HES to diagnosis of and median survival after diagnosis of a hematologic malignancy were compared between those with HES and HE. Kaplan-Meier curves and Log rank test were used to compare survival in these two groups.

**Results:** Of the 2642 patients identified with HE or HES, 17 (median age 71.2 [IQR: 58.7-77.9] years; 53% male, 47% female) subsequently developed a hematologic malignancy (frequency of 0.63%). Eight other patients were noted to have a malignancy that preceded the diagnosis of HE or HES and were excluded from this analysis. Of the 17 patients used in the analysis, 8 (median age 73.3 [68.0-77.9] years; 63% male) met the most recent consensus criteria for HES (47%), while the other 9 (median age 68.7 [55.7-77.5] years; 44% male) met criteria for HE (53%). The average time from HES diagnosis to hematologic malignancy diagnosis was  $31.8 \pm 13.5$  months, and from HE diagnosis to hematologic malignancy diagnosis was  $5.4 \pm 2.7$  months (two-tailed, T-test  $p = 0.08$ ). Lymphomas developed in 10 of 17 patients (59%) while leukemias developed in 7 of 17 patients (41%). Peripheral T cell lymphomas occurred with the greatest frequency (29.4%). Cutaneous lymphomas (17.6%), chronic lymphocytic leukemia (17.6%) and eosinophilic leukemia (17.6%) were the next most common followed by one case each of Hodgkin's lymphoma, diffuse large B-cell lymphoma and acute myelogenous leukemia. Median survival from the time of malignancy diagnosis for patients with underlying HES was 29 months compared to 60 months for HE patients (Log-rank test  $p = 0.5$ ).

**Conclusions:** The development of hematologic malignancies among people diagnosed with HE or HES is a relatively rare event but may be higher than the general population. Hematologic malignancy tended to develop later in patients with HES compared to HE, with a trend toward earlier mortality, however, neither comparison was statistically significant. Our study may have underestimated frequency of hematologic malignancy in HE and HES, as patients who were initially identified but then lost to follow-up were not included in the analysis.



## POSTER 78

**EVALUATION OF EFFECTS OF PDGFR-BLOCKING TYROSINE KINASE INHIBITORS ON GROWTH AND MIGRATION OF NEOPLASTIC EOSINOPHILS IN FIP1L1/PDGFR<sup>A</sup>+ CHRONIC EOSINOPHILIC LEUKEMIA (CEL)**

Irina Sadovnik<sup>1</sup>, Els Lierman<sup>2,3</sup>, Harald Herrmann<sup>4</sup>, Barbara Peter<sup>4</sup>, Verena Suppan<sup>1</sup>, Gabriele Stefanzi<sup>1</sup>, Winfried Pickl<sup>5</sup>, Jan Cools<sup>2,3</sup>, Peter Vandenberghe<sup>2,3</sup>, Peter Valent<sup>1,4</sup>

<sup>1</sup>Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Austria; <sup>2</sup>Center for Human Genetics, KU Leuven, Leuven, Belgium, <sup>3</sup>Center for Human Genetics, University Hospital Leuven, Leuven, Belgium, <sup>4</sup>Ludwig Boltzmann Cluster Oncology, Vienna, Austria; and <sup>5</sup>Institute of Immunology, Medical University of Vienna, Austria.

**Background:** In chronic eosinophilic leukemia (CEL), the transforming oncoprotein FIP1L1-PDGFR<sup>A</sup> (F/P) is a major target of therapy. In most patients, the PDGFR<sup>A</sup>-targeting tyrosine kinase inhibitor (TKI) imatinib induces complete molecular remissions. For patients who are intolerant or resistant against imatinib, novel TKI may be alternative drugs. However, little is known about the effects of novel TKI on growth and survival of neoplastic human eosinophils.

**Methods:** In the current study, we examined the *in vitro* effects of 12 kinase blockers on growth and viability as well as cytokine-induced migration of EOL-1 cells, a human F/P+ eosinophilic cell line. In addition, we examined TKI effects on primary neoplastic eosinophils obtained from a patient with F/P+ CEL, one with aggressive systemic mastocytosis and eosinophilia (ASM-eo) and one with reactive hypereosinophilia (HE).

**Results:** In EOL-1 cells, major growth-inhibitory effects were seen with all PDGFR<sup>A</sup>-blocking agents, with IC<sub>50</sub> values in the low nM-range: ponatinib: 0.1-0.2 nM, sorafenib: 0.1-0.2 nM, masitinib: 0.2-0.5 nM, nilotinib: 0.2-2 nM, dasatinib: 0.5-2 nM, sunitinib: 1-2 nM, and midostaurin: 5-10 nM. These drugs were also found to block the activity of PDGFR-downstream signaling molecules, including Akt, S6, and STAT5 in EOL-1 cells. All effective TKI produced dose-dependent apoptosis in EOL-1 cells as determined by microscopy, Annexin-V/PI-staining, and staining for active caspase-3. Next, we applied the most effective TKI on primary neoplastic eosinophils. In these experiments, ponatinib, dasatinib, and nilotinib were found to suppress growth of eosinophils obtained from a patient with F/P+ CEL and one with ASM-eo (IC<sub>50</sub> <0.5 μM). In the patient with reactive HE, the TKI also produced growth inhibition, but IC<sub>50</sub> values were higher compared to neoplastic eosinophils. We also examined drug effects on growth of Ba/F3 cells expressing the imatinib-resistant F/P mutants T674I and D842V. In these experiments, sunitinib was found to inhibit the growth of Ba/F3 cells expressing the T674I mutant of F/P. However, strong inhibitory effects on both mutants were only seen with ponatinib. We then examined the effects of various TKI on stroma cell-derived factor-1 (SDF-1)-induced migration of neoplastic eosinophils. We found that imatinib, nilotinib, dasatinib, ponatinib, sorafenib, and masitinib inhibit SDF-1-induced migration of EOL-1 cells in a dose-dependent manner (effective range: 10-100 nM). Finally, we analyzed TKI effects on expression of activation-linked cell surface antigens on EOL-1 cells. In these experiments, ponatinib and sorafenib were found to downregulate expression of CD25 and CD63 in EOL-1 cells, whereas the other TKI showed no effects.

**Conclusions:** In summary, our data show that various novel TKI counteract growth, survival, activation, and migration of neoplastic human eosinophils. The most potent agent that also blocks all known mutant-forms of F/P appears to be ponatinib. Novel PDGFR-targeting TKI, such as ponatinib, may be attractive alternative drugs for the treatment of imatinib-resistant or intolerant CEL.

**POSTER 79****THE WHO CLASSIFICATION OF MASTOCYTIC DISEASE DOES NOT INCORPORATE DISEASE SUCH AS ETV6-ABL1: A DISTINCT GENETIC GROUP**

Finella C. Brito-Babapulle

Eosinophilia is a common accompaniment of mastocytic diseases. The 2008 World Health Organization (WHO) classification of systemic mastocytosis (SM) recognizes 4 varieties. In 2009 Pardanani et al thought it was clinically more useful to consider specific entities, such as SM-MPN, systemic mastocytosis with chronic myelomonocytic leukemia, SM-MDS, and systemic mastocytosis with-acute leukemia, rather than their broad reference as SM-AHNMD and stated in 2011 that “ it has its limitations”

A 30 year old female presented at 30 weeks pregnancy with circulating myeloid blasts in the blood but a normal full blood count. Bone marrow aspirate revealed a “dry” tap but trephine biopsy showed sheets of eosinophils and mastocytic cells but only 5% which marked with CD117 and CD2 and were loosely distributed through the marrow. Marked reticulin fibrosis and osteosclerosis was present. A trephine roll showed the presence of 23% hypergranulated basophilic cells more than seen in the aspirate. These were not obvious on trephine assessment. An ABL1- ETV6 fusion was identified While being observed and on treatment with imatinib the platelet count and haemoglobin fell with an increasing blast count and treatment for acute leukaemia as per the AML 17 protocol has been started. She did not have the D8176V mutation.

This case does not easily fit into the classification given above as the acute transformation is not an associated disease but a direct consequence of it.

This case may have been classified as an eosinophilic disorder rather than mastocytic due to the small number of mast cells. Our best description of the case would be that it is a chronic mastocytic disorder in transformation to an acute myeloid leukaemia analogous to blast transformation of CML.

## POSTER 80

## CLOZAPINE INDUCED EOSINOPHILIA WITH PERIMYOCARDITIS

Urs Steiner<sup>1,2</sup>, Patrick Loretan<sup>1</sup>, Beat Voegtli<sup>1</sup>

<sup>1</sup> Spitalnetzbern Tiefenau, Department of internal Medicine, Berne, Switzerland

<sup>2</sup> Spitalnetzbern Ziegler, Department of Internal Medicine, Division of Allergology and Clinical Immunology, Berne, Switzerland

**Background:** Clozapine, is an atypical antipsychotic drug with strong affinity for C4-dopaminergic receptors and potent serotonergic, noradrenergic, histaminic and cholinergic receptor blocking ability. It is the acknowledged treatment of choice for patients with severe treatment-refractory schizophrenia. Clozapine may cause several adverse effects of which the most severe ones are granulocytosis, thromboembolism and severe cardiovascular side effects including cardiomyopathy and myocarditis. Clozapine induced perimyocarditis is life threatening and may lead to sudden cardiac death. The assumed underlying pathomechanism is a hypersensitivity reaction but until now this is not shown.

We present the history of a young man with clozapine induced hypereosinophilia with perimyocarditis and discuss steps for investigating the pathomechanism of this life threatening adverse drug reaction.

**Method and case description:** A 20 years old man was treated with Clozapine because of a refractory schizophrenia. 14 days after treatment onset he developed fever up to 40°, nausea and diarrhea. In the further course sinus tachycardia and hypotension occurred. Hospitalisation was necessary. Clinical and laboratory exams didn't show any sign of infection. No skin reaction. Then the patient developed heart failure with pulmonary edema. Intensive care medicine was necessary. Clozapine was stopped. Laboratory exams revealed eosinophilia with a maximum of 1.75G/l and high sensitivity Troponin (hsT) was elevated with 0.42mg/l (<0.014). Electrocardiogram showed unspecific T- wave and ST- segment abnormalities. Transthoracic echocardiography showed a small pericardial effusion, diastolic dysfunction, slightly reduced left ventricular function (EF 50%) and abnormal texture of the myocardium, compatible with perimyocarditis. Skin Tests and Lymphocyte transformation tests (LTT) are under investigation.

**Results:** After withdrawal of clozapine and medical treatment for heart failure, the patient recovered well in a few days. Transthoracic echocardiography seven days later was normal. Eosinophilia and hsT normalized slowly during the following six weeks. The results of the investigations to elucidate the pathomechanism of an assumed hypersensitivity reaction to Clozapine with Lymphocyte transformation test and skin tests are not yet available.

**Conclusion:** Perimyocarditis is a rare but frequently fatal side effect of the anti-psychotic drug clozapine. The onset is most often early after treatment initiation. The underlying pathomechanism of this life threatening adverse drug reaction is not understood yet. We assume a drug induced T-cell reaction. Drug specific T-cells initiate polyclonal eosinophilia. Therapy is withdrawal of the culprit drug. Because glucocorticosteroids may provoke an acute psychosis, they are reserved for severe cases.

Eosinophilia is a common aspect in adverse drug reactions e.g. maculopapular exanthema or drug related eosinophilia with systemic symptoms (DRESS). An effort should be made to isolate and to characterise drug specific T-cells and specially investigate their cytokine production. This could give a clue about how and why eosinophils are proliferating, get activated and are capable of homing in different organs where they may cause life threatening damage.

**POSTER 81****IS PERIPHERAL BLOOD EOSINOPHILIA BEING INVESTIGATED APPROPRIATELY? A RETROSPECTIVE REVIEW OF 89 PATIENTS**

MJ York, Institute for Lung Health, United Kingdom; AJ Wardlaw, Institute for Lung Health, United Kingdom

**Background:** Peripheral blood eosinophilia can be due to variety of causes including parasitic infestation, myeloproliferative disease, Churg Strauss Syndrome, severe asthma, fungal airway disease and drug hypersensitivity. We suspect that a large number of patients with elevated eosinophil counts are not investigated appropriately for this. This study assessed whether patients with a recorded persistent eosinophilia were correctly identified and referred for investigation at the specialist Hypereosinophilia (HES) clinic in Glenfield Hospital, Leicester.

**Method:** This was a retrospective case note review of 89 patients who had a peripheral blood eosinophilia greater or equal to  $1 \times 10^9/L$  recorded in February 2012 at the University Hospitals of Leicester.

**Results:** 110 patients were recorded by the haematology department as having an eosinophil count of  $> 1.0 \times 10^9/L$  during February 2012. We were able to obtain the notes of 89 of these patients. 45/89 patients had a persistently raised eosinophil count (greater or equal to  $1 \times 10^9/L$ ) over a 6 month period prior to February 2012. Of these, 7/45 had been assessed in the specialist HES clinic in Leicester.

Lymphoproliferative disease was diagnosed in 10/89, 4/10 of whom had also received haemodialysis. Respiratory disease had been diagnosed in 7/89 patients; Churg Strauss Syndrome in 1/7, severe asthma in 5/7 and fungal airway disease in 1/7. Chronic parasitic infection was diagnosed in 1/89, malignancy in 8/89, miscellaneous HES clinic diagnoses in 3/89 and allergic disease in 4/10. In total, 12/89 patients had received haemodialysis. There was no clear cause for eosinophilia in 48/89 patients.

**Conclusions:** Hypereosinophilia can denote severe life-threatening disease and should be investigated to determine the cause. The fact that only a minority of patients with peripheral eosinophilia were referred to a specialist clinic for a full investigation is of potential concern. Increased awareness by healthcare professionals regarding the significance and importance of investigating eosinophilia is required.

**POSTER 82****CAUSES OF AN EOSINOPHILIA PRESENTING TO A SPECIALIST HYPEREOSINOPHILIC CLINIC**

MJ York, Institute for Lung Health, United Kingdom; AJ Wardlaw, Institute for Lung Health, United Kingdom

**Background:** A marked peripheral blood eosinophilia is relatively uncommon in the UK. The eosinophil count is often ignored, resulting in delays in diagnosis and effective treatment. Several hypereosinophilic conditions are potentially life threatening.

**Methods:** In 2003 a specialist clinic to diagnose and manage people with an unexplained and persistent peripheral blood eosinophilia ( $>1 \times 10^9/L$ ), was established at Glenfield Hospital Leicester, a cardiorespiratory hospital serving a population of 1.0 million. Patients were investigated according to a structured algorithm.

**Results:** 171 patients have been seen since 2003. Most referrals were from other consultants. 29 patients were diagnosed with hypereosinophilic syndrome of which 7 were regarded as having the myeloproliferative variant, two with documented FIP1L1-PDGFRalpha mutation disease. The commonest single cause of a raised eosinophil count (33), was infection with helminthic parasites. The commonest organ affected was the respiratory tract (Churg-Strauss 12; Fungal airway disease 23; Eosinophilic pneumonia 15; severe asthma 28). Drug allergy was diagnosed in 10 cases and malignancy in 4. No diagnosis was achieved in 7 patients. There was a wide range of eosinophil counts in each diagnostic category with myeloproliferative HES having the highest mean ( $26 \times 10^9/L$ ) and individual ( $50 \times 10^9/L$ ) count. The mean and maximum count for severe asthma was 3.4 and  $5.3 \times 10^9/L$ , respectively.

**Conclusions:** The cause of a raised eosinophil count can be found in most people by following a structured algorithm. FIP1L1-PDGFRalpha disease is very rare affecting 1:250,000 of the population. The level of peripheral blood eosinophilia is not particularly helpful in guiding the diagnosis although uncomplicated asthma is very unlikely if the count is greater than  $5 \times 10^9/L$ . The bias towards respiratory and parasite disease in the clinic probably reflects the location of the service and the ethnicity of the population.

## POSTER 83

**UNEXPECTED CYTOKINE PROFILES SUGGEST DIFFERENTIAL ROLES OF EOSINOPHILOPOIETIN-PRODUCING T CELLS IN PERIPHERAL BLOOD EOSINOPHILIA AND HYPEREOSINOPHILIA**

Christina Stoeckle and Hans-Uwe Simon

Institute of Pharmacology, University of Bern, Switzerland

**Background:** The cytokines IL-5, IL-3 and GM-CSF play important roles in eosinophil development, survival and function. In order to better understand their role in eosinophilia of unknown cause, we investigated expression of these cytokines in peripheral blood T cells as well as plasma levels of these cytokines of these patients.

**Methods:** Intracellular cytokine staining was performed on healthy donor and patient PBMC and plasma cytokine levels measured by ELISA.

**Results:** As expected, some of these patients showed an increased proportion of IL-5- or IL-3- producing CD4<sup>+</sup> T cells. However, in a significant proportion of patients, IL-5 producing CD8<sup>+</sup> T cells, so called Tc2 cells, which can only be detected at very low levels in healthy donors, were prominent. Interestingly, IL-3-producing CD4<sup>+</sup> T cells were elevated in eosinophilic, but not hypereosinophilic patients. Most surprising, however, was a reduction of GM-CSF-producing CD4<sup>+</sup> T cells in patients with hypereosinophilia.

**Conclusion:** While increased IL-5 and/or IL-3 plasma levels and production by peripheral blood T cells could be observed in many patients, T cell-derived GM-CSF did not appear to play a role in reactive eosinophilia in our patients. The selective increase in IL-3-producing CD4<sup>+</sup> T cells in eosinophilic compared to normal and hypereosinophilic patients may suggest that the underlying mechanisms responsible for elevated eosinophil levels might differ between eosinophilia and hypereosinophilia. In addition to CD4<sup>+</sup> T helper cells, CD8<sup>+</sup> T cells also need to be considered as a potential eosinophilopoietin source in reactive eosinophilia.

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## POSTER 84

**INVASIVE CUTANEOUS TRICHOPHYTON RUBRUM INFECTION ASSOCIATED WITH BLOOD AND TISSUE EOSINOPHILIA IN THE SETTING OF A CARD9 DEFICIENCY.**

Florence Roufousse<sup>1</sup>, Jean-Christophe Goffard<sup>1</sup>, Liliane Schandené<sup>2</sup>, Véronique del Marmol<sup>3</sup>, Frédérique Jacobs<sup>4</sup>.

Department of Internal Medicine<sup>1</sup>, Laboratory of Immunology<sup>2</sup>, Department of Dermatology<sup>3</sup>, Department of Infectious Disease<sup>4</sup>, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium.

**Background:** Invasive *Trichophyton Rubrum* (TR) infection is uncommon and is generally observed in the setting of immunodepression. *Trichophyton Rubrum* antigens have been shown to elicit either immediate hypersensitivity (IH) or delayed-type hypersensitivity reactions, depending on the host<sup>1</sup>. Immediate hypersensitivity responses are associated with chronic or recurrent infections and increased TR-specific IgE levels. Although increased serum IgE indicates an antigen-specific Th2-type response, hypereosinophilia has been reported only once in the setting of chronic and invasive TR infection<sup>2</sup>.

We report the case of a 38-year old male patient of North African origin with severe invasive TR cutaneous infection that progressed despite administration of numerous systemic anti-mycotic agents, and in whom a novel *CARD9* missense mutation\* was shown to be responsible for altered *CARD9* function. He consistently had increased blood eosinophil counts, reaching 4300/ml, marked eosinophilic infiltrates in infected skin, as well as a marked elevation of serum IgE levels (5380 U/ml). Hypereosinophilia likely contributed to intense pruritis and to the development of an intracardiac thrombus.

*CARD9* mutations have been detected in patients with chronic mucocutaneous candidiasis<sup>3</sup>, but hypereosinophilia has not been reported in this setting, arguing against a causal link between congenital *CARD9* deficiency and hypereosinophilia.

We hypothesized that the chronic hypereosinophilia observed in our patient was the consequence of a Th2-driven immune response against TR antigens.

**Methods:** Serum IgE antibodies against TR and *Candida Albicans* (CA) -derived antigens were assessed by routine RAST, and a basotest was performed on whole blood in presence of TR and CA extracts. Phospho-STAT3 induction in lymphocytes following *in vitro* culture with IFN $\alpha$  was explored using flow cytometry. Isolated PBMC were stimulated with phorbol ester (PMA) and an ionophore (A23187) for 4 hours to assess intracytoplasmic cytokine expression within CD4 T cells; cytokine expression was also assessed after 96h incubation in presence of TR antigen alone, followed by polyclonal restimulation with PMA+A23187.

**Results:** TR-specific IgE were detected in patient serum with both RAST and basotest methods (9.2 U/ml and 96,7% CD63+ basophils respectively), whereas both tests were negative for CA. IFN $\alpha$ -induced lymphocyte phospho-STAT3 expression was comparable to healthy control. Polyclonal T-cell stimulation showed that a small subset (2%) of CD4 T-cells co-expressed Th2 cytokines (IL-4, IL-5, and IL-13) together with intense IL-2 expression. However, short-term culture with TR antigen alone didn't elicit Th2 cytokine expression within CD4 T-cells.

**Conclusion:** We report the case of a patient who developed a "reactive" hypereosinophilic syndrome, secondary to chronic invasive TR infection in the setting of congenital *CARD9* deficiency. Previous studies have shown that TR infection may exacerbate atopic disease in patients who develop an IH response to TR-derived antigens<sup>4</sup>. The presence of TR-specific IgE in our patient and a small circulating subset of clear-cut Th2-like CD4 T-cells suggests an antigen-specific Th2 immune response may be driving hypereosinophilia, a hypothesis that is currently being explored in experimental conditions designed to favor outgrowth of TR-responsive cells.

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\* The *CARD9* mutation was demonstrated and characterized in the Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSEM U980, Necker Medical School in Paris. A manuscript describing *CARD9* mutations associated with deep dermatophytosis in 17 patients (including the one described above) has recently been accepted for publication in the *New England Journal of Medicine* (first author F. Lanternier).

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A

**Ackerman, Steven**, University of Illinois at Chicago, Chicago, IL, sackerma@uic.edu

**Akuthota, Praveen**, Beth Israel Deaconess Medical Center, Boston, MA, pakuthot@bidmc.harvard.edu

**Alam, Rafeul**, National Jewish Health, Denver, CO, United States, AlamR@NJHealth.org

**Andersson, Jennie**, University of Gothenburg, Gothenburg, Sweden, jennie.andersson@microbio.gu.se

**Andersson, Kerstin**, Göteborg University, Göteborg, Sweden, kerstin.andersson@microbio.gu.se

**Appleton, Judith**, Baker Institute for Animal Health, Ithaca, NY, jaa2@cornell.edu

**Arita, Makoto**, University of Tokyo, Tokyo, Japan, marita@mol.f.u-tokyo.ac.jp

---

B

**Bafadhel, Mona**, ILH, Leicester, London, United Kingdom, mona.bafadhel@yahoo.com

**Bandeira de Melo, Christianne**, Universidade Federal Do Rio de Janeiro, Rio de Janeiro, Brazil, cbmelo@biof.ufrj.br

**Baruch- Morgenstern, Netali**, Tel Aviv University, Tel Aviv, Israel, netalimorg@gmail.com

**Bebbington, Christopher**, Allakos Inc., San Mateo, CA, United States, cbebbington@allakos.com

**Bettigole, Sarah**, Harvard University, New York, NY, United States, sab2051@med.cornell.edu

**Blom, Kristin**, Uppsala University, Uppsala, Sweden, kristin.blom@medsci.uu.se

**Bochner, Bruce**, Johns Hopkins University School of Medicine, Baltimore, MD, bbochner@jhmi.edu

**Boix, Ester**, Universitat Autònoma de Barcelona, Cerdanyola Del Valles, Spain, ester.boix@uab.cat

**Bozik, Michael**, Knopp Biosciences, Pittsburgh, PA, United States, michael.bozik@knoppbio.com

**Bozza, Patricia**, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, pbozza@ioc.fiocruz.br

**Broide, David**, UC San Diego, La Jolla, CA, United States, dbroide@ucsd.edu

**Buels, Kalmia**, Beth Israel Deaconess Medical Center, Boston, MA, United States, kbuels@bidmc.harvard.edu

**Busse, William**, University of Wisconsin Medical School, Madison, WI, United States, ww@medicine.wisc.edu

**Butterfield, Joseph**, Mayo Clinic, Rochester, MN, United States, butterfield.joseph@mayo.edu

---

C

**Cheon, In Su**, Seoul National University, Seoul, Korea, ciss80@snu.ac.kr

**Chu, Derek**, McMaster University, Hamilton, ON, Canada, chudk@mcmaster.ca

**Chu, Van Trung**, Deutsches Rheuma Forschungszentrum, Berlin, Germany, vantrung.chu@mdc-berlin.de

**Cook-Mills, Joan**, Northwestern University, Chicago, IL, United States, j-cook-mills@northwestern.edu

**Costanza, Rino**, Chiesi LTD, Cheadle, Cheshire, United Kingdom, rcostanza@chiesi.uk.com

**Cross, Nick**, ncpc@soton.ac.uk

---

D

**Davoine, Francis**, University of Alberta, Edmonton, AB, Canada, fdavoine@ualberta.ca

**Delbeke, Marie**, Inserm U995- Université de Lille 2, Lille, France, marie0delbeke@gmail.com

**Denburg, Judah**, McMaster University, Hamilton, ON, Canada, denburg@mcmaster.ca

**Diny, Nicola**, Johns Hopkins University, Baltimore, MD, United States, ndiny@jhsph.edu

**Doyle, Alfred**, Mayo Clinic Arizona, Scottsdale, AZ, United States, doyle.alfred@mayo.edu

**Driss, Virginie**, U 995 Inserm University Lille2, Lille, United States, France, virginie.driss@yahoo.fr

**Dworetzky, Steven**, Knopp Biosciences, Pittsburgh, PA, United States, dworetzky@knoppbio.com

**Dyer, Kimberly**, NIH - IIS/LAD/NIAID, Bethesda, MD, United States, kdyer@niaid.nih.gov

---

E

**Enav, Benjamin**, Pediatric Gastroenterology of Northern Virginia, Burke, VA, United States, ben@enav.us

**Eriksson, Ann-Katrin**, Diagnostics Development, Uppsala, Sweden, ann-katrin@diagnosticsdevelopment.com

**Esnault, Stephane**, University of Wisconsin, Madison, WI, United States, sesnault@wisc.edu

**Ethier, Caroline**, University of Alberta, Edmonton, AB, Canada, cethier@ualberta.ca



# PARTICIPANT LIST

## F

---

**Fahy, John**, john.fahy@ucsf.edu

**Farahi, Neda**, University of Cambridge, Cambridge, United Kingdom, nf231@cam.ac.uk

**Fillon, Sophie**, University of CO - Denver, Aurora, CO, United States, sophie.fillon@ucdenver.edu

**Flamand, Nicolas**, Université Laval, Québec City, QC, Canada, nicolas.flamand@criucpq.ulaval.ca

**Fosbury, David**, Teva Pharmaceuticals, Frazer, PA, United States, Hugh.fosbury@tevapharm.com

**Frei, Robert**, Medical University of Graz, Graz, Austria, robert.frei@medunigraz.at

**Fryer, Allison**, Oregon Health and Science University, Portland, OR, United States, allison.fryer@gmail.com

## G

---

**Gao, Fan Kimberly**, University of Illinois at Chicago, Chicago, IL, United States, fgao4@uic.edu

**Gatault, Solène**, U995 inserm University Lille 2, Lille, France, solene.gatault@hotmail.fr

**Gleich, Gerald**, University of Utah, Salt Lake City, UT, United States, gerald.gleich@hsc.utah.edu

## H

---

**Heinemann, Akos**, akos.heinemann@medunigraz.at

**Helbig, Grzegorz**, Silesian Medical University, Katowice, Poland, ghelbig@o2.pl

**Hogaboam, Cory**, hogaboam@med.umich.edu

**Hui, Claudia**, McMaster University, Hamilton, ON, Canada, claudia.ck.hui@gmail.com

## J

---

**Jacobsen, Elizabeth**, Mayo Clinic Arizona, Scottsdale, AZ, United States, jacobsen.elizabeth@mayo.edu

**Jacoby, David**, Oregon Health & Science University, Portland, OR, United States, jacobyd@ohsu.edu

**Johansson, Mats**, University of Wisconsin, Madison, WI, United States, mwjohansson@wisc.edu

**Jönsson, Ulla-Britt**, Uppsala University, Uppsala, Sweden, ulla-britt.jonsson@medsci.uu.se

## K

---

**Kahn, Jean Emmanuel**, Hopital Foch, Suresnes, France, je.kahn@hopital-foch.org

**Kay, A. Barry**, Imperial College, London, London, United Kingdom, a.b.kay@imperial.ac.uk

**Khoury, Paneez**, National Institutes of Health, Takoma Park, MD, United States, khouryp@niaid.nih.gov

**Kita, Hirohito**, Mayo Clinic Rochester, Rochester, MN, United States, kita.hirohito@mayo.edu

**Kiwamoto, Takumi**, Johns Hopkins University School of Medicine, Baltimore, MD, United States, t-kiwamoto@jhmi.edu

**Klion, Amy**, NIAID/NIH, Baltimore, MD, United States, amy@zunkeler.com

**Koenderman, Leo**, UMCU, Utrecht, Netherlands, l.koenderman@umcutrecht.nl

**Kolbeck, Roland**, MedImmune LLC, Gaithersburg, MD, United States, kolbeckr@medimmune.com

**Kurosky, Alexander**, University of Texas Medical Branch, Galveston, TX, United States, akurosky@utmb.edu

## L

---

**Lacy, Paige**, University of Alberta, Edmonton, AB, Canada, paige.lacy@ualberta.ca

**Lampinen, Maria**, Uppsala University, Uppsala, Sweden, maria.lampinen@medsci.uu.se

**Laviolette, Michel**, IUCPQ (Hopital Laval), Quebec, QC, Canada, michel.laviolette@med.ulaval.ca

**Lee, James**, Mayo Clinic Arizona, Scottsdale, AZ, United States, jjlee@mayo.edu

**Legrand, Fanny**, NIAID NIH, Washington DC, DC, United States, fanny.legrand@nih.gov

**Leiferman, Kristin**, University of Utah Health Care, Salt Lake City, UT, United States, kristin.leiferman@hsc.utah.edu

**Levi-Schaffer, Francesca**, The Hebrew University of Jerusalem, Jerusalem, Israel, francescal@ekmd.huji.ac.il

**Lingblom, Christine**, Biomedicine, Gothenburg, Sweden, christine.lingblom@microbio.gu.se

**Liu, Linying**, Beth Israel Deaconess Medical Center, Boston, MA, United States, lliu6@bidmc.harvard.edu

**Luna, Tatiana**, UFRJ, Rio de Janeiro, Brazil, luna@biof.ufrj.br

## M

**Matthews, John**, Genentech, Inc, South San Francisco, CA, United States, matthews.john@gene.com

**McKenzie, Andrew**, anm@mrc-lmb.cam.ac.uk

**Melo, Rossana**, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil, rossana.melo@ufjf.edu.br

**Minnicozzi, Michael**, NIH - NIAID, Bethesda, MD, United States, minnicozzim@niaid.nih.gov

**Miyata, Jun**, University of Tokyo, Tokyo, Japan, junmiyata@hotmail.co.jp

**Mjösberg, Jenny**, Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden, jenny.mjosberg@ki.se

**Moshkovits, Itay**, Tel-Aviv University, Tel Aviv, Israel, itaymoshko@gmail.com

**Munitz, Ariel**, Tel Aviv University, Tel Aviv, Israel, arielm@post.tau.ac.il

## N

**Nair, Parameswaran**, McMaster University, Hamilton, ON, Canada, parames@mcmaster.ca

**Nesi, Renata**, Harvard University - USA / Federal University of Rio de Janeiro - Brazil, Boston, MA, United States, rnesi@bidmc.harvard.edu

**Neves, Josiane**, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, josiane.sabbadini.neves@gmail.com

**Nizankowska-Mogilnicka, Ewa**, Jagiellonian University Medical College, Krakow, Poland, ewan@ghml.pl

## O

**Ogbogu, Princess**, Ohio State University, Columbus, OH, United States, princess.ogbogu@osumc.edu

**Ortega, Hector**, GlaxoSmithKline, Research Triangle Park, NC, United States, hector.g.ortega@gsk.com

## P

**Patel, Pallavi**, Imperial College London, United Kingdom, p.patel10@imperial.ac.uk

**Pease, James**, Imperial College London, London, United Kingdom, j.pease@imperial.ac.uk

**Peinhaupt, Miriam**, Medical University of Graz, Graz, Austria, miriam.peinhaupt@medunigraz.at

**Percopo, Caroline**, NIAID/NIH -> IIS/LAD/NIAID/NIH, Bethesda, MD, percopoc@niaid.nih.gov

**Philipson, Richard**, GlaxoSmithKline, Cambridge, United Kingdom, Richard.S.Phippison@gsk.com

**Porter, Linsey**, University of Cambridge, Cambridge, United Kingdom, ljp54@medschl.cam.ac.uk

**Prakash Babu, Senbagavalli**, National Institutes of Health, Bethesda, MD, United States, prakashs2@niaid.nih.gov

**Price, David**, University of Aberdeen, Cambridge, United Kingdom, shuna@rirl.org

**Prussin, Calman**, NIAID/NIH, Rockville, MD, United States, cprussin@niaid.nih.gov

## R

**Radonjic-Hoesli, Susanne**, Institute of Pharmacology, University of Bern, Bern, Switzerland, susanne.hoesli@pki.unibe.ch

**Raible, Donald**, RaibleD@MedImmune.com

**Roberts, Nerys**, Chelsea & Westminster and Gread Ormond St Hospital, London, United Kingdom, n.roberts@doctors.net.uk

**Rosenberg, Helene**, NIAID/NIH, Bethesda, MD, United States, hrosenberg@niaid.nih.gov

**Rothenberg, Marc**, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, rothenberg@cchmc.org

**Roufousse, Florence**, Hôpital Erasme (ULB), Brussels, Belgium, froufoss@ulb.ac.be

**Rubin, Jenny**, Uppsala University, Uppsala, Sweden, jenny.rubin@medsci.uu.se

## S

**Sadovnik, Irina**, Medical University of Vienna, Vienna, Austria, irina.sadovnik@meduniwien.ac.at

**Samarasinghe, Amali**, University of Tennessee Health Science Center, Memphis, TN, United States, asamaras@uthsc.edu

**Shamri, Revital**, Beth Israel Deaconess Medical Center, Brookline, MA, United States, rshamri@bidmc.harvard.edu

**Shik, Dana**, Tel Aviv, Israel, danashik@gmail.com

**Simon, Dagmar**, University Hospital Bern, Bern, Switzerland, dagmar.simon@insel.ch

**Simon, Hans-Uwe**, University of Bern, Bern, Switzerland, hus@pki.unibe.ch

**Singh, Anish**, Empire Clinic, Leederville WA, Australia, anish@empireclinic.com.au

# PARTICIPANT LIST

**Spencer, Lisa**, Beth Israel Deaconess Medical Center, Boston, MA, United States, lspencer@bidmc.harvard.edu

**Steiner, Urs**, Spitalnetz Bern, Berne, Switzerland, uc.steiner@bluewin.ch

**Stoeckle, Christina**, University of Bern, Bern, Switzerland, christina.stoeckle@pki.unibe.ch

**Straumann, Alex**, Swiss EoE Research Network, Olten, Switzerland, alex.straumann@hin.ch

**Sturm, Eva**, Medical University of Graz, Graz, Austria, eva.sturm@medunigraz.at

**Sullivan, Mary**, Knopp Biosciences, Pittsburgh, PA, United States, mary@knoppbio.com

## T

---

**Teo, Pearlina**, Astar Singapore, Singapore, Singapore, dapearl@gmail.com

**Tomasevic, Nenad**, Allakos, Foster City, CA, United States, ntomasevic@allakos.com

## U

---

**Ueki, Shigeharu**, Akita University, Akita, Japan, ueki-shige@nifty.com

## V

---

**Vaillancourt, Marc**, Novartis Pharma, Dorval, QC, Canada, marc.vaillancourt@novartis.com

**Valent, Peter**, Medical University of Vienna, Vienna, Austria, peter.valent@meduniwien.ac.at

**van Der Merwe, Rene**, Medimmune LTD, Cambridge, United Kingdom, vandermerwer@medimmune.com

**Vial, Catherine**, University of Leicester, Leicester, United Kingdom, cv12@le.ac.uk

**Von Gunten, Stephan**, University of Bern, Bern, Switzerland, stephan.vongunten@pki.unibe.ch

## W

---

**Wang, Haibin**, Beth Israel Deaconess Medical Center, Billerica, MA, United States, hwang1@bidmc.harvard.edu

**Wang, Yui-Hsi**, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, yui\_hsi.wang@cchmc.org

**Wardlaw, Andrew**, Leicester University, Leicester, United Kingdom, aw24@leicester.ac.uk

**Watts, Colin**, University of Dundee, Dundee, United Kingdom, c.watts@dundee.ac.uk

**Wechsler, Michael**, National Jewish Health, Denver, CO, United States, mikewechsler@gmail.com

**Weller, Peter**, Beth Israel Deaconess Medical Center, Boston, MA, United States, pweller@bidmc.harvard.edu

**Wenneras, Christine**, Sahlgrenska University Hospital, Goteborg, Sweden, christine.wenneras@microbio.gu.se

**Wicher, Sarah**, Oregon Health & Science University, Portland, OR, United States, wicher@ohsu.edu

**Willetts, Lian**, University of Alberta, Edmonton, AB, Canada, willetts.lian@mayo.edu

**Williams, Timothy**, Imperial College London, London, United Kingdom, tim.williams@imperial.ac.uk

**Wilson, Shona**, Dept. Pathology, Uni. of Cambridge, Cambridge, United Kingdom, sw320@cam.ac.uk

**Wong, Tina**, Mayo Clinic, Rochester, MN, United States, wong.tina@mayo.edu

**Wright, Adam**, NIHR respiratory BRU, Leicester, United Kingdom, aw287@le.ac.uk

## Y

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**Yamada, Kelsey**, National Institutes of Allergy and Infectious Diseases, Bethesda, MD, United States, kelsey.yamada@nih.gov

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**Zimmermann, Nives**, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States, zimn0@cchmc.org

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