

# *Final Program*

11<sup>TH</sup> BIENNIAL SYMPOSIUM OF THE  
INTERNATIONAL EOSINOPHIL SOCIETY

# *Portland*

**OREGON**

9-13 JULY 2019



## Join JLB in Commemorating the IES 2019 Conference!

The Journal of Leukocyte Biology will publish a special issue featuring articles related to IES 2019 Conference. Both reviews and reports of original research are welcomed! All articles will be peer-reviewed according to the high standards of the JLB. Please mention the meeting in your cover letter to be sure your article is targeted to this special issue. If submitting a review, please send a brief description of the topic to the editors prior to writing: [jlbstaff@leukocytebiology.org](mailto:jlbstaff@leukocytebiology.org) (this will ensure topics do not overlap). Questions? Email [jlbstaff@leukocytebiology.org](mailto:jlbstaff@leukocytebiology.org).

Submit by November 1, 2019 to be a part of this special-focus issue!

**JLB** JOURNAL OF  
LEUKOCYTE  
BIOLOGY



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# Table of Contents

|                                |    |
|--------------------------------|----|
| Welcome .....                  | 3  |
| Acknowledgements .....         | 4  |
| Exhibit Hall Information ..... | 5  |
| General Information .....      | 6  |
| Social Activities.....         | 8  |
| IES Scientific Program .....   | 9  |
| Awards .....                   | 18 |
| Abstracts .....                | 22 |

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# Welcome



On behalf of the leadership at IES, I am excited to invite you to Portland, Oregon for our meeting from 9-13 July 2019. Portland is situated at the confluence of the Willamette (will-am-et) River as it flows north to join the Columbia River. Portland has three snowcapped volcanoes visible to the north and east, and a mountain range separating it from the Pacific Ocean to the west. Within a 90 minute drive, you can be hiking in the Columbia Gorge, walking on the Pacific beaches, or sipping pinot in the Willamette Valley. Portland itself is highly walkable, but also has excellent public transportation. The meeting will be held at the Marriott Waterfront Downtown, right along the Willamette River and park, in the center of the city. In between the fabulous talks and posters I hope you are able to catch up with colleagues while enjoying Powell's Books, the Saturday Market, the Rose Garden, Chinese Garden and Japanese Gardens as well as the many public parks and wonderful restaurants. I look forward to welcoming you to my city!

On behalf of the organizing committee.

Allison Fryer  
Scientific Program Director

Bruce Bochner  
IES President

# Acknowledgements

IES thanks the following partners for their support of the 11<sup>th</sup> Biennial Symposium

## Gold Supporters



## Silver Supporter



## Bronze Supporters



## Additional Support

Novartis Institutes for BioMedical Research, Inc.

## Society Sponsors



## Grant Support

### National Institutes of Health

Funding for this conference was made possible [in part] by 1 R13 AI147390-01 from 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.

# Exhibit Hall Information

## Booth 100 GlaxoSmithKline

5 Moore Drive  
Research Triangle Park, North Carolina, 27709  
www.us.gsk.com

GSK is a science-led global healthcare company with a mission to help people do more, feel better, and live longer. We research, manufacture and make available a broad range of medicines, vaccines and consumer healthcare products. Visit our exhibit for information about our products and resources.

## Booth 200 Miltenyi Biotec Inc.

6125 Cornerstone Court East  
San Diego, CA 92121  
www.miltenyibiotec.com

Miltenyi Biotec provides products that advance biomedical research and cellular therapy. Our innovative tools support research from basic research to translational research to clinical application. Our more than 25 years of expertise includes immunology, stem cell biology, neuroscience, and cancer. Miltenyi Biotec has more than 1,700 employees in 25 countries.

## Booth 300 GlaxoSmithKline

5 Moore Drive  
Research Triangle Park, North Carolina, 27709  
www.us.gsk.com

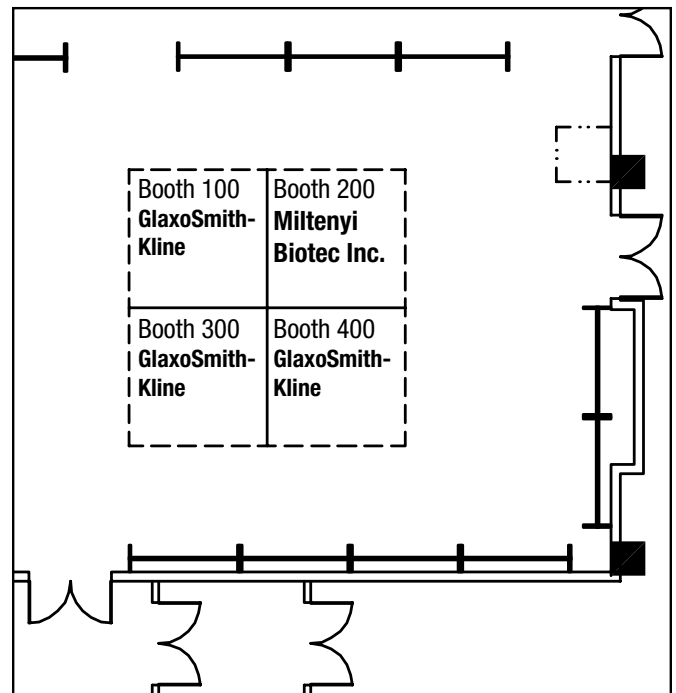
GSK is a science-led global healthcare company with a mission to help people do more, feel better, and live longer. We research, manufacture and make available a broad range of medicines, vaccines and consumer healthcare products. Visit our exhibit for information about our products and resources.

## Booth 400 GlaxoSmithKline

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## Exhibit Hall Map



Please allow time in your schedule to visit the exhibits located in the poster hall.

### EXHIBITOR HOURS:

Wednesday, 10 July  
17:00 – 19:00

Thursday, 11 July  
17:00 – 19:00



# General Information

The 11<sup>th</sup> Biennial Symposium of the International Eosinophil Society (IES) will be held in Portland, Oregon, USA. Portland, Oregon's largest city, sits on the Columbia and Willamette rivers, in the shadow of snow-capped Mount Hood. It's known for its parks, bridges and bicycle paths, as well as for its eco-friendliness and its microbreweries and coffeehouses. Iconic Washington Park encompasses sites from the formal Japanese Garden to Oregon Zoo and its railway. The city hosts thriving art, theater and music scenes.

## Currency

The currency used in the USA is the Dollar (USD). There are ATMs widely available for cash withdrawal. Credit cards are also accepted at most hotels, restaurants and shops.

## Electricity

The standard electrical voltage in the USA is 120 Volts and 60 Hertz.

## Language

The official language of the 11<sup>th</sup> Biennial Symposium is English.

## Time Zone

Portland, Oregon is in the Pacific Daylight Time Zone (GMT-7), which is seven hours behind of the Coordinated Universal Time (UTC).

## Tipping

While tipping is not mandatory in most of the United States, it is customary in many circumstances for service, especially at almost all sit-down restaurants which offer table service and many food servers depend on tips as an essential part of their wage.

## Breakfast with a Mentor Session

These sessions are geared towards young scientists at the beginning of their career. Sessions will be in an informal setting where scientists will share with young investigators some of what they have experienced and learned in their career thus far.

Space is limited; please check at the registration desk for open slots.

07:00 – 08:00 AM Wednesday, 10 July - Dr. Jackie Wirz

07:00 – 08:00 AM Thursday, 11 July - Dr. Lisa Wheatley

07:00 – 08:00 AM Thursday, 11 July - Dr. Michael Minnicozzi

## Ask An Expert

We are introducing a new session that will focus on protocols and methodologies. Eosinophils are tricky cells to work with. Beginning right after their isolation from biological samples to their identification and use in downstream in vitro assays-eosinophils are not the friendliest of cells. This session identifies certain "eosinophil biologists" who have expertise in eosinophil-related methodologies and allows them to share their expertise with the attendees. Don't forget to follow us @EosinophilSoc and #AskAnEosExpert and #IES2019.

### Ask An Expert Session 1, Wednesday 10 July 17:00 – 19:00

#### 17:00 – 18:00

- E1 - A Functional Ex Vivo Human Esophageal Mucosal Explant System for Eosinophilic Esophagitis – Seema Aceves, USA
- E2 - Eosinophils and Influenza Virus: How to Make the Introductions - Amali Samarasinghe, USA
- E3 - Eosinophil-Specific Reagents, Tools, and Techniques – Elizabeth Jacobsen, USA

#### 18:00 – 19:00

- E4 - Use of Flow Cytometry to Identify and Isolate of Mouse Eosinophils - Caroline Percopo, Albert Sek, and Helene Rosenberg, USA
- E5 - Interrogating Signaling Pathways in Primary Human Eosinophils - Jeremy O'Sullivan, USA
- E6 - Quantitative 3D Confocal Microscopy Illuminates Eosinophil-Nerve Interactions in Airways – Matthew Drake, and Alexandra Pincus, USA

### Ask An Expert Session 2, Thursday 11 July 17:00 – 19:00

#### 17:00 – 18:00

- E7 - Isolation and Identification of Extracellular Vesicles from Human Eosinophils – Praveen Akuthota, USA
- E8 - Isolation of Human Eosinophils from Whole Blood - Kiho Son, Canada
- E9 - Methods for Enumeration and Measuring Functional Responses of Eosinophil Progenitor Cells - Roma Sehmi, Canada
- E10 - Development and Utility of Eosinophil Granule Protein Measurement in Blood and Body Fluids - Michelle Makiya, USA

#### 18:00 – 19:00

- E11 - How to Visualize ETosis-Mediated Eosinophil Extracellular Traps - Shigeharu Ueki, Japan
- E12 - Activation Markers of Eosinophils by Flow Cytometry: Examples from an EoE Study – Mats Johansson, USA
- E13 - Eosinophil Image Analysis and Degranulation Assays – Paige Lacy and Sarah Almas, Canada



# General Information

## Travel Arrangements and Airport Transfers

The airport is located 9 miles (14.5 km) northeast of downtown Portland and is conveniently connected to the city center via MAX light rail train.

### LIGHT RAIL

The MAX light rail Red Line is the easiest way to travel to and from the airport. Here are some quick facts:

- The trip between the airport and downtown Portland takes about 38 minutes.
- An adult ticket costs \$2.50 (Youth \$1.25, Honored Citizen \$1). MAX ticket machines return change in coins, so small bills are recommended.
- You can roll your luggage on board.
- The first train of the day arrives at PDX at 4:45 a.m. The last train departs PDX at 11:50 p.m.
- The MAX station and ticket machines are located on the lower level, next to the south baggage claim area (turn right at the base of the escalator).

For complete schedules and more information, visit [www.trimet.org/schedules/maxredline.htm](http://www.trimet.org/schedules/maxredline.htm).

### TAXICABS

The average taxi fare from the airport to downtown is approximately \$35 before gratuity. Radio Cab offers a discount with coupons available on their website. The one-way trip takes 20-40 minutes. You can find taxis waiting in the center section of the airport terminal's lower roadway outside of baggage claim; to get back to the airport from the city center, you can order a cab through the city's main operators or from a hotel with a dedicated taxi stand.

### SHUTTLES

When you leave the airport, you will find the shuttles at the bottom entrance of the terminal (outside the baggage claim area) on the other side of the second strip ("Island 2") near the parking garage.

## Venue

The 11<sup>th</sup> Biennial Symposium of IES will be held at the Portland Marriott Waterfront Downtown. 1401 SW Naito Pkwy. Portland, OR 97201

## Weather

The average temperature in July is between 80°F/27°C and 57°F/14°C.

# Social Activities

## Wednesday, 10 July

### NETWORKING EVENT FOR GRADUATE STUDENTS AND POST-DOCS

Wednesday 7:15pm (after posters!)

meet at the Marriott Hotel Registration desk.

Come join us for a fun and walkable evening out in Portland! We will be exploring the downtown area, starting with the indoor food carts of Pine Street Market and then heading to Ground Kontrol for drinks and games. All trainees welcome!

## Thursday, 11 July

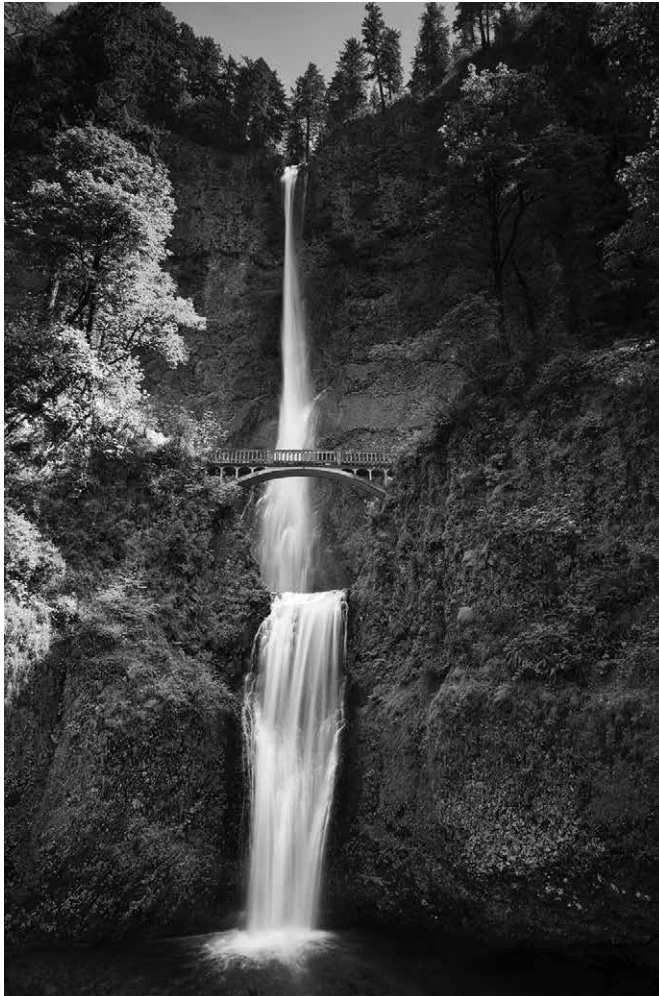
### TRIP TO MULTNOMAH FALLS

There will be a trip for those interested out to Multnomah Falls leaving from the hotel at 12:45, and will return in time to attend the Ehrlich Award Winner Lecture that is scheduled for 16:00 start.

Availability information can be found at the registration desk.

Cost: \$20.00

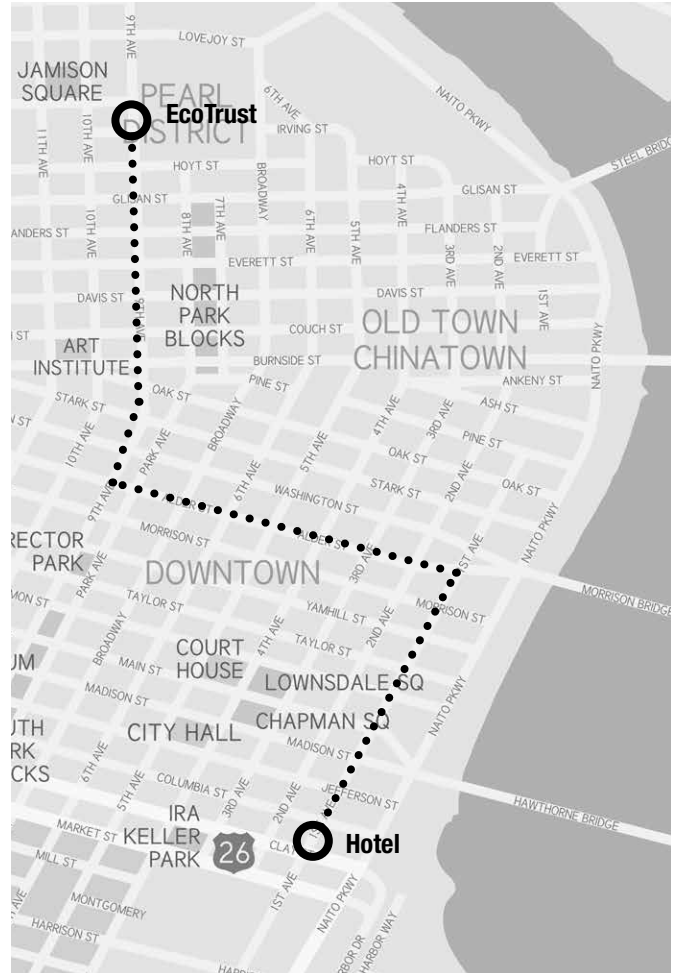
*(An optional box lunch will be available to purchase, \$60.00)*



## Friday, 12 July

### TASTE OF OREGON NETWORKING AND DINNER EVENT

There will be an off-site networking event taking place at the Jean Vullum Natural Capital Center at the EcoTrust Building from 18:00 - 19:45, with dinner to follow at the Portland Marriott Waterfront Hotel starting at 20:30.



*Above is the walking route from the hotel to EcoTrust. 1.4 miles one way, approximately 28 minutes.*

Another option to use would be the MAX Green Line and MAX Yellow line of the Portland transit system, with the station located 8 blocks away from the Marriott (corner of 6th & Madison) and ending 5 blocks away from the EcoTrust Building (corner of 6th & Hoyt)

These events will be available to all attendees to take part in. If you would like to bring a guest, please stop by registration to purchase a ticket.

# Scientific Program


## Tuesday, 9 July 2019

All Scientific Sessions to take place at Portland Marriott Waterfront Downtown in room Salon E, Level 1, unless otherwise noted

### Welcome (Portland Marriott Downtown Waterfront)

- 18:00 - 18:30 Welcome  
*Bruce Bochner, USA & Allison Fryer, USA*
- 18:30 - 19:00 History of Eosinophil Discovery or Why Did Eosinophils Capture My Attention  
*Jerry Gleich, USA*
- 19:00 - 21:00 **Welcome Reception**  
*Location: Truss/ Mt. Hood Room, Level 2*

## Wednesday, 10 July 2019

- 07:00 - 08:00 **Breakfast with a Mentor Session:**   
How to Navigate a Scientific Conference  
*Jackie Wirz, USA*  
*Location: Salem Room, Level 1*

### Session 1: Eosinophils Across Species: What Does That Tell Us About Their Physiological Role, Evolution and Choice of Animal Models

*Moderators: Joan Cook-Mills, USA and Elizabeth Jacobsen, USA*

- 08:15 - 08:45 On the Origin of Eosinophils by Means of Natural Selection: Preservation of a Favored Leukocyte in the Struggle for Life  
*Steve Ackerman, USA*
- 08:45 - 09:15 Eosinophils and Disease Models from Mouse to Man: We are Stronger Together  
*Helene Rosenberg, USA*
- 09:15 - 09:45 Eosinophil Identity in the Tissue Contexts of Development, Homeostasis, and Disease  
*Sergejs Berdnikovs, USA*
- Abstract Presentations:**
- 09:45 - 10:00 Real-Time in Vivo Imaging of Tissue Resident Eosinophils under Homeostatic and Inflammatory Conditions  
*Kamala D. Patel, Canada*

- 10:00 - 10:30 **Morning Break**

### Session 2: Eosinophil Life Cycle, Hematopoiesis and Recruitment, Activation and Death

*Moderators: Dagmar Simon, Switzerland and Peter Weller, USA*

- 10:30 - 11:00 Hemopoietic Mechanisms in Eosinophilic Inflammation  
*Roma Sehmi, Canada*
- 11:00 - 11:30 Eosinophils in Liver Death  
*Todd Davidson, USA*
- 11:30 - 12:00 Eosinophil ETosis and DNA Traps: A New Look at Eosinophilic Inflammation  
*Shigeharu Ueki, Japan*
- 12:00 - 12:30 Eosinophil Shape Change and Granule Secretion  
*Paige Lacy, Canada*
- 12:30 - 13:00 **IES Business Meeting**
- 13:00 - 14:30 **Lunch (on your own)**
- 14:30 - 15:30 **Gleich Award Winner Lecture:**  
Eosinophil Depletion Suppresses Radiation-Induced Small Intestinal Fibrosis  
*Naoki Takemura, Japan*
- 15:30 - 16:00 Eosinophil Extracellular DNA Traps in Skin Diseases  
*Dagmar Simon, Switzerland*

# Scientific Program

Wednesday, 10 July 2019

## Abstract Presentations:

- 16:00 - 16:15 First Ultrastructural Demonstration of Galectin-10 in Resting and Activated Human Eosinophils  
*Rossana Melo, Brazil*
- 16:15 - 16:30 Siglec-8 Signals through an Unanticipated Set of Molecules to Induce Integrin Upregulation and Activation, ROS Production, and Cell Death in Eosinophils  
*Jeremy O'Sullivan, USA*

## 17:00 - 19:00 Poster Session 1, Location: Room - Salon F & I, Level 1

### Ask An Expert Session 1

- 17:00 – 18:00 E1 - A Functional Ex Vivo Human Esophageal Mucosal Explant System for Eosinophilic Esophagitis  
*Seema Aceves, USA*
- E2 - Eosinophils and Influenza Virus: How to Make the Introductions  
*Amali Samarasinghe, USA*
- E3 - Eosinophil-Specific Reagents, Tools, and Techniques  
*Elizabeth Jacobsen, USA*
- 18:00 – 19:00 E4 - Use of Flow Cytometry to Identify and Isolate of Mouse Eosinophils  
*Caroline Percopo, Albert Sek, and Helene Rosenberg, USA*
- E5 - Interrogating Signaling Pathways in Primary Human Eosinophils  
*Jeremy O'Sullivan, USA*
- E6 - Quantitative 3D Confocal Microscopy Illuminates Eosinophil-Nerve Interactions in Airways  
*Matthew Drake, and Alexandra Pincus, USA*
- Poster 1 Eosinophil Infiltration Does Not Exacerbate Muscle Pathology in the MDX Mouse Model of Duchenne Muscular Dystrophy  
*Albert Sek*
- Poster 3 In Vitro and In Vivo Inhibition of Eosinophil Activation by Lysophosphatidylcholine and Synthetic Alkyl-Lysophosphatidylcholine  
*Eva Knuplez*
- Poster 5 Characterizing SIGLECF+GR1- and SIGLECF+GR1+ Mouse Bone Marrow-Derived Eosinophils (bmEos) Cultured Ex Vivo  
*Eric Mai*
- Poster 9 Nasal Polyps as a Presenting Sign of Systemic Eosinophilic Diseases  
*Yossi Rosman*
- Poster 11 Dexpramipexole Responsiveness is increased in Eosinophilic Patients  
*Calman Prussin*
- Poster 13 Single Site, Five-Year Experience with Human Eosinophil Isolation by Density Gradient Centrifugation and Immunomagnetic Negative Separation  
*Yun Cao*
- Poster 15 Genetic Variation in Surfactant Protein-A2 and the Effect on Eosinophil Resolution in Allergic Airways  
*Alane Blythe C. Dy*
- Poster 17 Eosinophil Cytolysis on IgG Requires Microtubule Array Formation, and p38 Phosphorylation Downstream of Reactive Oxygen Species Production  
*Stephane Esnault*
- Poster 19 IL-9 and IL-13 Storage and Release from Eosinophils  
*Sarah Almas*
- Poster 21 The Role of Eosinophils in Obesity-Related Asthma  
*Gina Calco*
- Poster 23 Kinetic Studies of Galectin-10 Release From Eosinophils to Proliferating T Cells  
*Christine Lingblom*
- Poster 25 Eosinophil Responses to IL-4, IL-33 and Bacteria are Differentially Regulated by Apoptotic Cells  
*Avishay Dolitzky*

# Scientific Program

Wednesday, 10 July 2019

- Poster 27      Harnessing Hymenolepis Diminuta Extract to Active Anti-Tumorigenic Activities of Eosinophils in Colorectal Cancer  
*Inbal Hazot*
- Poster 29      Granulocyte Kinetics in the Blood and Sputum of Patients with Eosinophilic and Neutrophilic Inflammation  
*Leo Koenderman*
- Poster 31      IgG4-Related Disease and Hypereosinophilic Syndrome: Overlapping Phenotypes?  
*Matthieu Groh*
- Poster 33      Allergic Bronchopulmonary Aspergillosis as a Complication of COPD: A New Entity?  
*Matthieu Groh*
- Poster 35      Intrinsic Sex-Specific Differences in Fibrotic Gene Expression and Pathology Contribute To Male Disease Predisposition in Pediatric Eosinophilic Esophagitis  
*Loan Duong*
- Poster 37      Urine Levels of Eosinophil Granule Proteins; A Better Biomarker of Eosinophilic Disease Activity?  
*Michelle Makiya*
- Poster 39      Fluid Shear Stress Induces Rapid Actin Rearrangement in Eosinophils  
*Kiho Son*
- Poster 41      Kruppel-Like Factor 3 (KLF3) Regulates Adipose Tissue Beiging via Eosinophils  
*Emily Vohralik*
- Poster 43      Association of Blood Eosinophil Phenotypes and Patient Self-Assessment Data with Response to Treatment in Eosinophilic Esophagitis  
*Sofie Albinsson*
- Poster 45      Eosinophilic Gastrointestinal Disease Treatment Approaches are Associated with Frequency of Feelings of Isolation  
*Pablo Albonia*
- Poster 47      Human Eosinophils Express a Distinct Gene Expression Program in Response to IL-3 Compared To Common Beta-Chain Cytokines IL-5 and GM-CSF  
*Praveen Akuthota*
- Poster 49      Eosinophils Suppress TH1 Responses and Restrict Bacterially Induced Gastrointestinal Inflammation  
*Isabelle Arnold*
- Poster 51      Clinical and Endoscopic Features of the Fibrostenotic Phenotype in Eosinophilic Esophagitis  
*Paul Fichtinger*
- Poster 53      Molecular, Endoscopic, Histological and Circulating Biomarker-Based Diagnosis of Eosinophilic Gastritis: Cross Sectional Multi-Site Study  
*Tetsuo Shoda*
- Poster 57      Eosinophils Alter Steady-State Small Intestinal IL-1 $\alpha$  and IL-1 $\beta$  Levels but are not Required for the Maintenance of Secretory IGA nor for Control of an Enteric Bacterial Pathogen  
*Rachael Brown*
- Poster 59      Transgenic Expression of a Novel Secreted Active Form of IL-33 Promotes Eosinophilia in a Mouse Model of Eosinophilic Esophagitis  
*Benjamin Wright*
- Poster 61      Hypereosinophilia Associated with Clonal CD3-CD4+ T Cells: Characterization and Outcome of a New Cohort of Patients  
*Sylvain Verbanck*
- Poster 63      CD200R1 is Upregulated in the Esophageal Epithelium in Eosinophilic Esophagitis  
*Netali Ben Baruch-Morgenstern*
- Poster 65      Eosinophil Subtype Specific Metabolic Activities  
*Sergei I. Ochkur*
- Poster 67      Eosinophils Modulate ILC2 Activities  
*Elizabeth Jacobsen*
- Poster 69      Inhibition of Eosinophil Transendothelial Migration by 5HTP Stimulation of HTR1A and HTR1B in Endothelial Cells  
*Joan Cook-Mills*
- Poster 71      PIN1 Regulates IL-5 Induced Eosinophil Polarization and Migration  
*Zhong-Jian Shen*





# Scientific Program

## Wednesday, 10 July 2019

- Poster 73 Adipose Tissue Eosinophils in Cancer-Associated Cachexia  
*Patrick Lenehan*
- Poster 75 Pharmacodynamic And Clinical Efficacy Data from Patient Sputum Subgroups in DREAM Treated with Mepolizumab Across A 10-fold Dose Range  
*Charlene Prazma*
- Poster 77 Palmitoylation of Galectin-10 in Human Eosinophils  
*Haibin Wang*

## Thursday, 11 July 2019

- 07:00 - 08:00 **Breakfast with a Mentor Session:**   
How NIH Supports Clinical Trials  
*Lisa Wheatley, USA*  
*Location: Salem Room, Level 1*
- 07:00 - 08:00 **Breakfast with a Mentor Session:**   
Program Officers, Study Sections, RFA and PAs: Learn the language, protocols and best practices to interact with the NIH  
*Michael Minnicozzi, USA*  
*Location: Medford Room, Level 1*

### Session 3: Eosinophils and Host Defense

*Moderators: Isabelle Arnold, Switzerland and Lisa Spencer, USA*

- 08:00 - 08:30 When Eosinophils Meet Viruses  
*Amali Samarasinghe, USA*
- 08:30 - 09:00 Eosinophils Suppress Th1 Responses and Restrict Bacterially Induced Gastrointestinal Inflammation  
*Isabelle Arnold, Switzerland*
- Abstract Presentations:**
- 09:00 - 09:30 Eosinophil Phenotypes in Immune Pathways  
*Elizabeth Jacobsen, USA*
- 09:30 - 09:45 Single Cell RNA Sequencing of Inflammatory Tissue T Cells in Eosinophilic Esophagitis  
*Ting Wen, USA*
- 09:45 - 10:00 Identification of Eosinophil-Specific Gene-Enhancer Regions  
*Jennifer Felton, USA*
- 10:00 - 10:30 **Morning Break**

### Session 4: Eosinophils and Nerves

*Moderators: Richard Costello, Ireland and Manali Mukherjee, Canada*

- 10:30 - 11:00 Maternal Inflammation and Offspring Eosinophils regulate Airway Nerve Development  
*Katie Lebold, USA*
- 11:00 - 11:30 Eosinophils Promote Sensory Nerve Growth in Human Asthma  
*Matthew Drake, USA*
- 11:30 - 12:00 Eosinophils and Itch  
*David Jacoby, USA*
- Abstract Presentations:**
- 12:00 - 12:15 Asthma is Associated with Brain Neurodegeneration: A Possible Effect of Eosinophilic Inflammation?  
*William Busse, USA*
- 12:15 - 12:30 Characterization of a Mouse Model of Heart Disease in Hypereosinophilia  
*Nives Zimmermann, USA*
- 12:30 - 15:00 **Lunch (on your own)**

# Scientific Program

Thursday, 11 July 2019

## Session 5: Eosinophils and Metabolic Disease

Moderators: Zhenying Nie, USA and Helene Rosenberg, USA

15:00 - 15:30 Extreme IL-33-Dependent Eosinophil Accumulation in Adipose Tissue  
*Alyssa Hasty, USA*

### Abstract Presentations:

15:30 - 15:45 The Role of Adipose Tissue-Resident Eosinophils in Adipocyte Metabolism and Whole-Body Energy Homeostasis  
*Elena Anna De Filippis, USA*

15:45 - 16:00 Eosinophils in Beige Adipose Tissue Activation  
*Kate Quinlan, Australia*

16:00 - 17:00 **Ehrlich Award Winner Lecture:**  
Regulation and Control of Eosinophilia  
*Marc Rothenberg, USA*

## 17:00 - 19:00 Poster Session 2, Location: Room - Salon F Level 1

### Ask An Expert Session 2

17:00 – 18:00 E7 - Isolation and Identification of Extracellular Vesicles from Human Eosinophils  
*Praveen Akuthota, USA*

E8 - Isolation of Human Eosinophils from Whole Blood  
*Kiho Son, Canada*

E9 - Methods for Enumeration and Measuring Functional Responses of Eosinophil Progenitor Cells  
*Roma Sehmi, Canada*

E10 - Development and Utility of Eosinophil Granule Protein Measurement in Blood and Body Fluids  
*Michelle Makiya, USA*

18:00 – 19:00 E11 - How to Visualize ETosis-Mediated Eosinophil Extracellular Traps  
*Shigeharu Ueki, Japan*

E12 - Activation Markers of Eosinophils by Flow Cytometry: Examples from an EoE Study  
*Mats Johansson, USA*

E13 - Eosinophil Image Analysis and Degranulation Assays  
*Paige Lacy and Sarah Almas, Canadian Assays*

Poster 2 Eosinophils from Normal Donors: How Do They Vary? How Do They Define Us?  
*Caroline Percopo*

Poster 4 Determining the Role of Eotaxin-1 in Eosinophil Development In Order To Promote Heterogeneity via an Ex Vivo Culture System  
*Ajinkya Limkar*

Poster 6 Eosinophilia and Hookworm Infection: A Living Drug for Autoimmune Diseases  
*David Pritchard*

Poster 8 Sustained Long-Term Efficacy and Safety of RPC4046, an Anti-Interleukin-13 Monoclonal Antibody, In Patients with Eosinophilic Esophagitis: Results from the Open-Label Extension of the Heroes Study  
*Cristian Rodriguez*

Poster 10 Markers of Esophageal Epithelial-Mesenchymal Transition (EMT) are Significantly Reduced in Active Eosinophilic Esophagitis Following 16 Weeks of Treatment with RPC4046, an Anti-Interleukin-13 Monoclonal Antibody  
*Cristian Rodriguez*

Poster 12 Differentiation and Activation of Eosinophils in the Human Bone Marrow at Steady State and During Experimental Acute Systemic Inflammation  
*Marwan Hassani*

Poster 14 Activated Eosinophils Promote an Anti-Tumorigenic Environment in Tumor Models of Colorectal Cancer  
*Melanie Kienzl*



# Scientific Program

Thursday, 11 July 2019

- Poster 16 Vitamin Deficiencies May Vary in Relation to Symptoms and Treatment Approaches for Eosinophilic Esophagitis  
*Pablo Albonia*
- Poster 18 Epidermal Expression of Eotaxins-1, 2, and 3 in Bullous Pemphigoid is Comparable to Other Eosinophil Rich Dermatoses: A Retrospective Immunohistochemical Analysis  
*Kyle Amber*
- Poster 20 Detection of Eosinophil Cytolytic Degranulation in Paraffin-Embedded Tissue  
*Mineyo Fukuchi*
- Poster 22 Human Eosinophils Express of Free Fatty Acid Receptor 2 And 3: Possible Implication of These Receptors in Interleukin-4 Production by Short Chain Fatty Acids  
*Myeong Seong Sim*
- Poster 24 Intraesophageal Administration of Oxazolone to Skin-Sensitized Mice Results In Experimental Eosinophilic Esophagitis  
*Shmuel Avlas*
- Poster 26 Human Neutrophils Release Extracellular DNA Traps in Response to Histoplasma Capsulatum Var. Capsulatum  
*Glaucia De A. Thompson De Souza*
- Poster 28 Regulation of Eosinophil Recruitment in Allergic Airway Inflammation by Tropomyosin Receptor Kinase A  
*Mythili Dileepan*
- Poster 30 Aspergillus Fumigatus-Induced Human Eosinophil Extracellular DNA Traps: Role of IP3K, p38 MAPK and Src Family  
*Marina Barroso*
- Poster 32 Improved Diagnosis of Asthma: Metabolomic Profiling of Urine  
*Darryl Adamko*
- Poster 34 AKST4290, a CCR3 Inhibitor, Is Efficacious In Models of Age-Related Eosinophilic Disorders  
*Arnaud Teichert*
- Poster 36 The TNF Superfamily Member 14 (TNFSF14/Light) Induces Pro-Inflammatory Responses in Fibroblasts in Eosinophilic Esophagitis  
*Mario Manresa*
- Poster 38 Lysophosphatidylserine Induces Eosinophil Extracellular Trap Cell Death  
*Hye Jeong Kim*
- Poster 40 The Thiazolidinediones as Potential Therapeutic Agents for Eosinophilic Esophagitis Pathologic Remodeling: A Preclinical Evaluation  
*Quan Nhu*
- Poster 42 Serum Eotaxin-1/CCL11 Levels Are Elevated in Severe Forms of Systemic Sclerosis  
*Guillaume Lefèvre*
- Poster 44 A Functional Role for Eosinophils in Host Resistance against Mycobacterium Tuberculosis  
*Katrin Mayer-Barber*
- Poster 46 Identification of Reactive Oxygen Species Production Site in Siglec-8 Induced Eosinophil Death  
*Toshihiro Tomii*
- Poster 48 Efficacy and Tolerance of Interferon-Alpha in Hypereosinophilic Syndromes. A Retrospective, Multicenter Study on 29 Patients  
*Jean Emmanuel Kahn*
- Poster 50 Eosinophil Subsets in Pediatric Exacerbation-Prone Asthma  
*Li Merlin*
- Poster 52 Eosinophil Associated Type-2 Gene Expression in Childhood Asthma  
*Matthew Altman*
- Poster 54 Improvement in Quality Of Life (QOL) In Pediatric Patients with Eosinophilic Esophagitis and Food Allergy after Initial Clinic Evaluation  
*Vibha Szafron*
- Poster 56 IL-33 Expression and Potential Activation of Eosinophils Is Associated with ORM DL3  
*Elizabeth Mckernan*
- Poster 58 Quantifying Mucosal Eosinophilic Inflammation in EOE Using the 1-Hour Esophageal String Test  
*Milica Grozdanovic*

# Scientific Program

## Thursday, 11 July 2019

- Poster 60 Elevated Serum Tarc in Patients with Idiopathic Hypereosinophilic Syndrome: A Marker of Lymphocyte- Driven Disease?  
*Julien Catherine*
- Poster 62 IL-13 Treated Esophageal Epithelial Cells Induce Eosinophil Survival and Activation  
*Julia Dunn*
- Poster 64 Effects of Sustained Eosinophil Depletion in Humans  
*Fei Li Kuang*
- Poster 66 Expression and Function of Type 1 and Type 2 Eosinophils  
*Christopher D. Nazaroff*
- Poster 68 Eosinophils Suppress Lung Allograft Rejection  
*Elizabeth Jacobsen*
- Poster 70 Early Life Represents a Vulnerable Time Window for IL-33-Induced Lung Pathology  
*Hirohito Kita*
- Poster 72 Multiple Roles of PIN1 in the Regulation of TLR7 Signaling in Eosinophils  
*Zhong-Jian Shen*
- Poster 74 Steroid Dose Reducing Effect of Benralizumab, a Monoclonal Antibody to IL-5Ra, In Eosinophilic Granulomatosis with Polyangiitis  
*Vamsi P. Guntur*
- Poster 76 Oral Corticosteroid Dose Modulation in Severe Asthma: Impact on Peripheral Blood Eosinophil Count  
*Charlene Prazma*
- Poster 78 Eosinophils Dampen Hepatic Ischemia Reperfusion Injury through Interleukine-33 Signaling  
*Jong-Min Jeong*

## Friday, 12 July 2019

### Session 6: Eosinophils in Chronic Inflammatory Disease - Asthma

*Moderators: William Busse, USA and Jeremy O'Sullivan, USA*

- 08:00 - 08:30 ASTHMA - Autoimmune Responses, Eosinophils and Complex Airway Disease  
*Manali Mukherjee, Canada*
- 08:30 - 09:00 ASTHMA - Anti IL5 Therapies for Severe Asthma: Targeting The Ligand vs The Receptor  
*Parameswaran Nair, Canada*
- 09:00 - 09:30 LUNG - Targeting Eosinophils in Eosinophil Granulomatosis with Polyangiitis  
*Michael E. Wechsler, USA*
- Abstract Presentations:**
- 09:30 - 09:45 Lung Eosinophils Increase Vagus Nerve Mediated Airway Reflex Bronchoconstriction in Mice  
*Zhenying Nie, USA*
- 09:45 - 10:00 Idiopathic Eosinophilic Vasculitis, Another Side of Hypereosinophilic Syndrome? A Comprehensive Analysis of 115 Cases in Asthma-Free and ANCA-Negative Patients  
*Guillaume Lefèvre, France*
- 10:00 - 10:30 **Morning Break**

# Scientific Program

Friday, 12 July 2019

## Session 7: Eosinophils in Chronic Inflammatory Disease- GI

Moderators: Seema Aceves, USA and Marc Rothenberg, USA

- 10:30 - 11:00 **ESOPHAGUS** - Eosinophilic Esophagitis-like Disease: Description of a New Disease Entity  
*Hans-Uwe Simon, Switzerland*
- 11:00 - 11:30 **GI OR SUBSETS** - Local Allergen Challenge Increases Tissue Eosinophil Populations at Remote Tissue Sites Along The Skin:Lung:Gut Axis and Primes for Exacerbated Allergic Reactions to New Antigens  
*Lisa Spencer, USA*
- 11:30 - 12:00 **GI** - Markers of Chronic Inflammatory Bowel Disease  
*Marie Carlson, Sweden*
- 12:00 - 12:30 **GI and SKIN** - A Pathophysiological Potpourri of Conundrums and Challenges in Eosinophil-Related Epithelial Inflammation  
*Kristin Leiferman, USA*

### Abstract Presentations:

- 12:30 - 12:45 Functional Role of Kallikrein 5 and Proteinase Activated Receptor (PAR)2 in Eosinophilic Esophagitis  
*Nurit Azouz, USA*
- 12:45 - 13:00 Efficacy of Rpc4046, an Anti-Interleukin-13 Monoclonal Antibody, In Patients with Active Eosinophilic Esophagitis: Analysis of the Steroid-Refractory Subgroup from the Heroes Study  
*Cristian Rodriguez, USA*

13:00 - 14:30 **Lunch** (on your own)

### CEGIR Lecture Session

(If you are interested in lunch please order from the registration desk by end of day Thursday, 11 July)

“Findings from the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR)”

- Introduction and Structure of CEGIR - *Marc Rothenberg, USA*
- Early Findings from the CEGIR EGID Observational Cohort - *Ikuo Hirano, USA*
- Partnering with Patients - *Seema Aceves, USA and Representatives from PAGS*
- Logistical Operations-Publications and Data Sharing - *Sandeep Gupta, USA*
- Training the Next Generation of EGID Leaders - *Quan Nhu, USA*
- NIAID Interests and Roles in Disease Consortia - *Lisa Wheatley, USA and Mike Minnicozzi, USA*
- Questions and Answers and Discussion

## Session 8: Eosinophils in Cancer

Moderators: Florence Roufosse, Belgium and Hans-Uwe Simon, Switzerland

- 15:00 - 15:30 Activated Eosinophils Exert Distinct Anti-Tumorigenic Activities in Colorectal Cancer  
*Ariel Munitz, Israel*
- 15:30 - 16:00 Charcot-Leyden Crystals as a Drugable Target in Type 2 Immunity  
*Bart Lambrecht, United States*

### Abstract Presentations:

- 16:00 - 16:15 Eosinophils Promote GM-CSF-Dependent Anti-Tumor Immunity Through Activation of Type I T-Cell Responses  
*Alessandra Gurtner, Switzerland*
- 16:15 - 16:30 Activated Eosinophil Subsets are an Integral Part of the Tumor Microenvironment in Lung Metastasis, Displaying Anti-Tumorigenic Activities  
*Sharon Grisaru, Israel*

# Scientific Program

Saturday, 13 July 2019

## Session 9: The Now and Future of Eosinophils as a Drug Target

Moderators: *Matthew Drake, USA and Fei Li Kuang, USA*

- 08:00 - 08:30 "Siglec"ting the Allergic Response for Therapeutic Targeting  
*Bruce Bochner, USA*
- 08:30 - 09:00 Developing Dexamipexole for the Treatment of Eosinophilic Disorders  
*Calman Prussin, USA*
- 09:00 - 09:30 Subtypes of Responders to Treatment in Hypereosinophilic Syndrome  
*Paneez Khoury, USA*
- 09:30 - 10:00 Eosinophil Depletion in Humans: Is It Safe?  
*Amy Klion, USA*
- 10:00 - 10:30 EOS, Exacerbations and Adherence... The Good, Bad and Ugly of Severe Asthma  
*Richard Costello, Ireland*
- Abstract Presentation:**
- 10:30 - 10:45 Alpha IIb integrin (CD41) on blood eosinophils is a potential biomarker for disease activity in eosinophilic esophagitis (EoE)  
*Mats Johansson, USA*
- 10:45 - 11:15 **Morning Break**
- 11:15 - 12:15 **Roundtable Discussion 1**  
New Emerging or Controversial Topics - Is There More Than One Type of Eosinophil?
- 12:15 - 13:15 **Roundtable Discussion 2**  
New Emerging or Controversial Topics - Eosinophils-What are They Good For?

## JLB Dedicated IES Meeting Issue Submit by November 1, 2019

Questions:  
[jlbstaff@leukocytebiology.org](mailto:jlbstaff@leukocytebiology.org)

**JLB** JOURNAL OF  
LEUKOCYTE  
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The *Journal of Leukocyte Biology* (JLB) will publish a special focus issue featuring articles related to topics of the IES 2019 meeting. Both review papers ("overviews") and original investigations are encouraged. All articles will be published online within weeks of acceptance, with the full "cluster" of articles published together in a special issue of JLB.



Submit your work for the dedicated issue by November 1, 2019 for the JLB July 2020 Issue. When submitting, be sure to note your paper is related to the IES 2019 Conference!

# Awards

## IES Travel Grant Recipients

IES was able to offer the ability to young and early career participants the chance to apply to be a Travel Grant recipient at the Biennial Symposium. Each applicant was asked to provide a short letter of application, a copy of their abstract, a letter of recommendation from a current member of IES and a copy of their Curriculum Vitae. Waived registration and a travel stipend were awarded to the following attendees:

Sofie Albinsson, Sweden  
Sarah Almas, Canada  
Shmulik Avlas, Israel  
Nurit Azouz, United States  
Marina Barroso, Brazil  
Julien Catherine, Belgium  
Mythili Dileepan, United States  
Avishay Dolitzky, Israel  
Julia Dunn, United States  
Loan Duong, United States  
Alane Blythe C. Dy, United States  
Sharon Grisar, Israel  
Milica Grozdanovic, United States  
Marwan Hassani, Netherlands  
Melanie Kienzl, Austria  
Ajinkya Limkar, United States  
Eric Mai, United States  
Mario Manresa, United States  
Quan Nhu, United States  
Albert Sek, United States  
Tetsuo Shoda, United States  
Kiho Son, Canada  
Glaucia Thompson, Brazil  
Sylvain Verbanck, Belgium

Travel Grant Recipients will be awarded with a certificate during the dinner event scheduled 12 July 2019.

# Awards

## Gleich Award

Dr. Naoki Takemura will receive the fifth Gerald J. Gleich prize to be awarded at the 11<sup>th</sup> Biennial Symposium of the International Eosinophil Society, Inc. in Portland, Oregon, United States. 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 J. 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 J. Gleich.

Naoki Takemura, Ph.D., is now a lecturer at Laboratory of Bioresponse Regulation, Graduate School of Pharmaceutical Sciences Osaka University, Japan. His long-standing interest has been in food, and therefore he was studying functional foods and gut physiology at School of Agriculture, Hokkaido University, Japan. He received his PhD from Graduate School of Life Science, Hokkaido University in 2010 for his study on the development of anti-obesity diet therapy using probiotics and prebiotics. During this research, he became strongly interested in gut immune system and started to work as a postdoc in the laboratory of Prof. Shizuo Akira at Osaka University in Japan, a world authority on research of innate immunity. Thereafter, he moved and served as an assistant professor at International Research and Development Center for Mucosal Vaccines, Institute of Medical Science, The University of Tokyo and Graduate School of Medicine, Chiba University in Japan. In his immunology research, he mainly studied the role of innate immunity in the development of radiation-induced gastrointestinal syndrome and also tried to develop new types of mucosal vaccines. He received several encouraging awards for his studies from domestic conferences (e.g. Japanese Association for Dietary Fiber Research, the Japanese Society for Immunology).



During his research about gut immunology, he got interested in small intestinal eosinophils, because their functions still remain almost unclear, despite being abundantly present in small intestine under normal conditions. Using pathological specimens of small intestines from humans and mice, he found their pivotal role in radiation-induced intestinal fibrosis, one of the most troublesome adverse effect in cancer patients received abdominal radiation therapy.

In March 2019, he took the current position to enlarge his research area to cell biology. He is now studying the relationship between dietary nutrition and innate immune responses by various cells in mucosal tissues, including eosinophils, other immune cells, and non-immune cells like epithelial cells.



**THE GERALD J. GLEICH AWARD  
INTERNATIONAL EOSINOPHIL  
SOCIETY, INC.  
2019**

*Naoki Takemura, PhD*

*In recognition of the most intriguing,  
high impact finding related to  
eosinophil biology published in the  
years 2017-2019.*





# Awards

## Ehrlich Lectureship

The Ehrlich Lectureship is awarded at the Biennial 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. The recipient this year is Dr. Marc Rothenberg.

Dr. Rothenberg is director of the Division of Allergy and Immunology at Cincinnati Children's Hospital Medical Center and the Bunning Professor of Pediatrics at Cincinnati Children's within the University of Cincinnati College of Medicine. He graduated summa cum laude with highest honors in chemistry and biochemistry from Brandeis University. He then completed the MD/PhD program at Harvard Medical School under Dr. Frank Austen, conducting studies on eosinophil hematopoiesis, as he developed the first culture system for human eosinophils. After completing residency at Children's Hospital, Boston, Dr. Rothenberg did a fellowship in allergy/immunology and hematology at Children's Hospital. Dr. Rothenberg did post-doctorate training with Dr. Philip Leder, Harvard Medical School, where he cloned the eotaxin chemokine. After being faculty at Harvard Medical School for one year, he came to the University of Cincinnati and Cincinnati Children's, where he has helped build a top program in research, and his division is a leader in allergy and immunology.

His research is focused on molecular analysis of allergic inflammation, primarily on the pathogenesis of eosinophilia. His laboratory takes a multi-disciplinary approach including the development of preclinical murine models: genetics, genomics, molecular immunology, and biochemistry. Dr. Rothenberg's awards include the 2007 E Mead Johnson Award from the Society of Pediatric Research, 2010 National Institutes of Health MERIT Award, being elected fellow of the American Association for the Advancement of Science American Society for Clinical Investigation, and recognition by Clarivate as a top 1% Highly Cited Researcher. His publications number over 400. He has served on review panels for journals/grant agencies including National Institutes of Health (NIH), Burroughs Trust, and Medical Research Council of the United Kingdom. He served for four years on the Advisory Council of National Institute of Allergy and Infectious Disease. He has been Associate Editor of the Journal of Allergy and Clinical Immunology since 2004. His research has been supported by sources including the NIH, Human Frontier Science Program Organization, Burroughs Wellcome Fund, Dana Foundation, Department of Defense, PCORI and the US-Israel Binational Fund.



**PAUL EHRLICH LECTURESHIP  
INTERNATIONAL EOSINOPHIL  
SOCIETY, INC.  
2019**

*Marc Rothenberg, MD, PhD*

***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.***



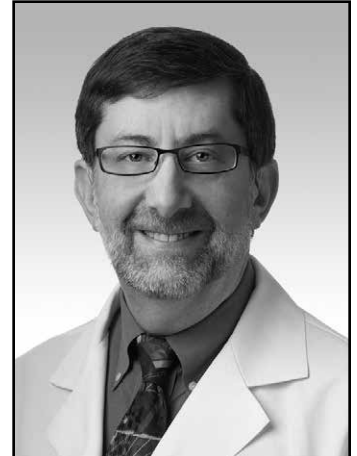


# Awards

## Service Award

The International Eosinophil Society, Inc., its leaders and members present to Dr. Bruce S. Bochner the distinguished Service Award both for his cardinal leadership with the International Eosinophil Society, Inc. and for his career-long contributions to innovative studies of the immunobiology of eosinophils.

Bruce S. Bochner, MD attended medical school at the University of Illinois College of Medicine in Chicago, and graduated with honors. After completing Internal Medicine residency training at the same institution, he began his postdoctoral allergy and immunology training at Johns Hopkins in the Division of Allergy and Clinical Immunology of the Department of Medicine, where he joined the faculty in 1988. In 1999, he became Professor of Medicine at Johns Hopkins, and from 2003-2013 was the Director of the Division of Allergy and Clinical Immunology. As of August 2013, Dr. Bochner moved to Chicago to become the Samuel M. Feinberg Professor of Medicine in the Division of Allergy and Immunology at the Northwestern University Feinberg School of Medicine. His primary clinical and NIH-funded research interests are on eosinophil and mast cell-related diseases and their treatment. His lab has a particular focus on the function of a receptor called Siglec-8 on these cells and he is a cofounder of a company called Allakos, Inc. that is actively testing anti-Siglec-8 therapies. He has been a member of the IES since its inception, has attended all but one IES meeting, hosted the 2015 IES meeting in Chicago, and has served in a number of IES leadership roles including its current president.



**SERVICE AWARD  
INTERNATIONAL EOSINOPHIL SOCIETY, INC.  
2019**

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*Bruce S. Bochner, MD*

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



# Invited Speaker Abstracts

## History of Eosinophil Discovery or Why Did Eosinophils Capture My Attention

Gerald Gleich

In 1965, I joined the Mayo Clinic in Rochester Minnesota, and one of my jobs was to care for hospitalized patients with asthma. It quickly became obvious that many of these patients had striking peripheral blood and sputum eosinophilia. I read the literature and found that lung tissues from patients dying of asthma showed dramatic eosinophil infiltration. With this background it seemed obvious to probe what the eosinophil is and what it does.

We isolated the eosinophil granule major basic protein (eMBP1) and determined that it is a potent toxin able to kill helminths, bacteria, mammalian cells and to activate many cells. eMBP1 was increased in sputa of patients with asthma, and eMBP1 instillation into monkey lungs caused bronchial hyperresponsiveness and bronchospasm. A recent study showed that eMBP1 instillation into the lungs of conscious mice stimulates cough. These findings argue that the eosinophil causes disease by releasing its toxic granule proteins.

We next localized eMBP1 in tissues from patients dying of asthma, suffering from helminth infections (onchocerciasis), various skin diseases, especially atopic dermatitis, eosinophilic cardiomyopathy and eosinophilic esophagitis (EoE). We discovered that eMBP1 is diffusely deposited on tissues and marks disease even when intact eosinophils are not evident. Studies of EoE show eMBP1 deposition at the site of strictures in the absence of intact eosinophils and correlating with EoE symptoms. Electron microscopy showed that 81% of eosinophils infiltrating esophageal tissue are dead, as defined by ruptured cytoplasmic membranes. These observations suggest that the eosinophil mediates tissue damage by disrupting and by releasing granules containing toxic granule proteins. The analogy to warfare is inescapable.

If eosinophils may not be present at the site of inflammation, then how can one be confident of identifying an eosinophil-related disease? Clearly, obtaining tissue and staining it for eMBP1 or another granule protein can be critical. We have recently completed experiments showing a new way to identify eosinophilic inflammation, namely by exposing EoE patients to radiolabeled heparin. In this presentation I will discuss results indicating that this is a robust method to identify eMBP1 deposition with the potential to reduce the frequency of endoscopy in EoE and to become a new standard for investigation of eosinophil-related diseases generally.”

## On the Origin of Eosinophils by Means of Natural Selection: Preservation of a Favored Leukocyte in the Struggle for Life

Steven J. Ackerman, PhD<sup>1</sup>, Nicole I. Stacy, DVM<sup>2</sup>, and Michael P. McGarry, PhD<sup>3</sup>

<sup>1</sup>University of Illinois at Chicago-College of Medicine, <sup>2</sup>University of Florida-College of Veterinary Medicine, and <sup>3</sup>MCG Consulting

The origins and evolution of the eosinophilic leukocyte have received only scattered attention since Paul Ehrlich first named this granulocyte for its eosin-staining acidophilic granules more than a century ago. The most recent consideration of this topic comes from a scholarly review by McGarry.<sup>1</sup> Cells in a variety of invertebrates may represent evolutionary precursors of modern-day vertebrate eosinophils. Links based on biochemical or genetic similarities are limited, but include the expression of the myeloperoxidases, of which EPX is eosinophil-specific. Studies suggest that myeloperoxidase and EPX diverged some 60-70 million years ago, but are not sufficiently robust to indicate when the earlier invertebrate to vertebrate evolution of the eosinophil lineage occurred.

Vertebrate eosinophils have been identified extensively in representative species, from fish to mammals, at the light/histologic, electron microscopic and biochemical levels.<sup>2</sup> Peroxidase-containing eosinophils have been definitively identified in embryonic and adult Zebra fish, which provide a potentially useful vertebrate model that can be genetically manipulated to study eosinophil development and functions.<sup>3</sup> Observations in the frog support a role for eosinophils in tissue remodeling events during metamorphosis (e.g., the shortening of the tadpole gut is accompanied by substantial infiltration of eosinophils), but the specific role of eosinophils in these complex metabolic, physiologic, and anatomical processes is undefined. Eosinophils are definitively present in most avian species, displaying very similar morphology to those in mammals. In the chicken, transcriptionally-regulated differentiation of eosinophil-committed progenitors to mature eosinophils is highly similar to humans.<sup>4</sup>

There are numerous published descriptions of mammalian eosinophils from guinea pig, hamster, rabbit, rat, bat, cat, dog, deer, cow, goat, horse, non-human primates including baboons, monkeys (Macaques), and of course multiple wild, hybrid, mutant and genetically altered (laboratory) strains of mice. Accumulating evidence in vertebrates, particularly in mammals, suggests that tissue-resident eosinophils function principally as regulators of “Local Immunity And/Or Remodeling/Repair” under both healthy and pathologic conditions, the so-called LIAR hypothesis,<sup>5</sup> elaborated by the late James J. Lee and colleagues, which makes a case for the principal function of eosinophils being the modulation of innate and adaptive immune responses, maintenance of tissue and metabolic homeostasis, and under pathologic conditions of tissue eosinophilia, inducers of remodeling and fibrosis. This is in contrast to their classically considered primary role in host defense against multicellular parasites and other pathogens. Although mammalian eosinophils are clearly well-equipped to kill and/or contain helminth parasites and their larval stages, their early appearance during evolution and accumulating studies of host immune

# Invited Speaker Abstracts

responses to helminths and other parasites in eosinophil-deficient mouse strains (e.g. PHIL, DdbiGATA, MBP-1<sup>-/-</sup>/EPX<sup>-/-</sup> double knock-out) strongly argue against this conferring a significant selective advantage in host defense during the evolution of the eosinophil.

The absence of significant, life-threatening developmental abnormalities or functional deficiencies in these eosinophil-deficient mouse strains, at least under the specific pathogen-free conditions present in most animal facilities, begs the question of why the eosinophil lineage continues to be ubiquitous in vertebrate species.

<sup>1</sup>McGarry MP. The evolutionary origins and presence of eosinophils in extant species. In: Lee JJ, Rosenberg HF, eds. Eosinophils in Health and Disease. Amsterdam: Elsevier, 2012:13-18.

<sup>2</sup>Stacy NI, Raskin RE. Reptilian eosinophils: beauty and diversity by light microscopy. *Vet. Clin. Pathol.* 2015;44:177-8.

<sup>3</sup>Balla KM, Lugo-Villarino G, Spitsbergen JM, et al. Eosinophils in the zebrafish: prospective isolation, characterization, and eosinophilia induction by helminth determinants. *Blood* 2010;116:3944-54.

<sup>4</sup>McNagny K, Graf T. Making eosinophils through subtle shifts in transcription factor expression. *J. Exp. Med.* 2002;195:F43-7.

<sup>5</sup>Lee JJ, Jacobsen EA, McGarry MP, et al. Eosinophils in health and disease: the LIAR hypothesis. *Clin. Exp. Allergy* 2010;40:563-75.”

## Eosinophils and Disease Models from Mouse to Man: We Are Stronger Together

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

Humans (*Homo sapiens sapiens*) and mice (*Mus musculus*) are closely-related species within the Animal Kingdom, as Primates and Rodents diverged as independent Orders only ~80 million years ago. While genetically defined mouse strains have been a mainstay of medical research since the 1950s, many researchers contend that mouse models have limited predictive reliability, and that clinically useful information will emerge only from direct exploration of human disease. At the same time, mouse models have virtually overtaken *in vivo* modeling, most notably since the development and ready availability of transgenic and gene deletion technologies.

With this in mind, we need to have clear and coherent means to evaluate mouse models of disease. As noted by Hau<sup>1</sup> in 2008, the ideal model permits the researcher to explore novel mechanisms, to explain complex interactions and/or to predict responses to treatment. He considers five categories of disease models, four of which can be directly applied to evaluation of mouse eosinophils and allergic airways disease: induced disease, genetic manipulation, spontaneous disease and negative disease. Allergen challenge, with or without systemic sensitization, is an example of induced disease<sup>2</sup>, in which a healthy mouse is subjected to a manipulation which results in Th2-cytokine mediated eosinophil recruitment to the lungs and airways. Allergen challenge studies in mice resulted in identification of molecular targets and contributed to the development of modern monoclonal antibody therapeutics.

At the same time, one needs to recognize and respect the limitations of individual studies. Notably, eosinophils from mice differ markedly from their human counterparts: they have distinctive cell surface markers and secretory proteins, they have a limited propensity to degranulate and have distinct responses to chemo-attractants. Nonetheless, generating humanized eosinophils with the transgenic *eoCre* strain<sup>3</sup> is now within reach. Beyond eosinophils, differences in anatomy and physiology between mouse and human lungs and airways are more difficult problem to overcome. The field in general might benefit from a larger appreciation of the complementary veterinary literature and the opportunity to study spontaneous disease in cats and horses.

Overall, it is important to focus not only on the conclusions from any one study, but on the design of and the limitations inherent in each model. There is no one experiment or trial that can fully reproduce the complexity of the human experience.

<sup>1</sup>Hau J. Chapter 1. *Sourcebook of Models for Biomedical Research*. 2008:3–8. <sup>2</sup>Rosenberg H.F., Druey, K. M. *J. Leukoc. Biol.* 2018;104:41-48. <sup>3</sup>Doyle A.D., et al. *J. Leukoc. Biol.* 2013;94:17-24.

**Funding:** NIAID DIR Z01-AI000941 to HFR.

## Hemopoietic Mechanisms in Eosinophilic Inflammation

Roma Sehmi

Asthma can be classified into various endotypes, the most clearly described being eosinophilic asthma. Eosinophils are a major contributor to physiological changes and airway re-modeling; treatments that control airway eosinophilia are associated with a decrease in symptoms and exacerbation rates. We postulate that {Sehmi, 2009 #829@author-year}{Sehmi, 2009 #829}airway eosinophilia arises

## Invited Speaker Abstracts

as a consequence of (i) recruitment of mature eosinophils from the periphery in response to locally elaborated chemoattractants and/or (ii) local maturation of bone marrow-derived eosinophil lineage-committed progenitors (EoP) within the lungs driven by locally elaborated eosinophilopoietic cytokines. {Sehmi, 2009 #829@@author-year}{Sehmi, 2009 #829} Our studies have shown that EoP, identified by flow cytometry and immunofluorescence microscopy as CD45+CD34+CD125+ cells are increased in asthmatic subjects compared to disease controls (COPD) and normal healthy subjects. The greatest number of EoP are found in sputum from prednisone dependent severe eosinophilic asthmatics (>3% eosinophilia), indicating an exaggerated eosinophilopoietic environment within the airways of these asthmatics. Targeting pathobiological pathways that promote tissue eosinophilia have provided effective control of eosinophilic asthma particularly in those patients refractory to the gold-standard of treatment, corticosteroids. Here we will present an over view of our findings from treatments targeting IL-5 or the IL-5 receptor and describe efficacy of the target and modality of treatments for attenuating *in-situ* eosinophilopoietic processes in severe asthmatics. In addition, we have shown that lung structural cells, namely smooth muscle cells, produce type 2 cytokines that can drive local differentiation of EoP. More recently, we report that primary bronchial epithelial cells from severe asthmatics spontaneously release TSLP which demonstrates clonogenic capacity and acts in synergy with IL-5 to promote eosinophil-lineage commitment and outgrowth from primitive precursors, *in vitro*. In this presentation, an overview of these findings will be discussed.

### Eosinophil Etosis and Extracellular Chromatin Traps: A New Look At Eosinophilic Inflammation

Shigeharu Ueki<sup>1</sup>, Peter F Weller<sup>2</sup>

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**Abstract:** Local turnover of eosinophils in diseased tissue mainly occurs through pathways other than apoptosis, i.e. cytolysis that generate abundant membrane-bound cell-free eosinophil granules. Our recent data indicated that cytolytic degranulation was mediated through an active form of cell death, namely extracellular trap cell death (ETosis). ETosis represents suicidal cell death originally found in human neutrophil (NETosis), involving development of sticky chromatin structures (neutrophil extracellular traps: NETs). In NETosis, granules are intracellularly disrupted before plasma membrane disintegration, thereby NETs are associated with various antimicrobial granule proteins such as myeloperoxidase. In contrast, intact granules are released extracellularly in the process of eosinophil ETosis, thereby eosinophil ETs (EETs) are often associated with cell-free granules. Compared with neutrophils, eosinophils contain far less proteases, and thus eosinophil chromatin structure is spared from endogenous proteolytic processing of nuclear histones. The stability of EETs pathologically contributes to the highly viscous character of eosinophil-dominant airway secretions. In addition, Charcot-Leyden crystal (CLC) formation, known as a classical evidence of eosinophilic inflammation, was closely associated with the process of eosinophil ETosis. Further studies on eosinophil cell death and postmortem activities will contribute to a novel perspective on various eosinophil-associated diseases.

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### When Eosinophils Meet Viruses

Amali Samarasinghe

The antediluvian notion that eosinophils in tissues are end stage cells that nonspecifically degranulate causing host pathology has been revisited in the past few decades in a variety of disease conditions. The potential for eosinophils to be an active member of host defense strategies against invading respiratory pathogens was discovered several decades ago and lately investigated in more depth. Although not specifically recruited as first responders during pulmonary viral infections, eosinophils can respond dynamically when exposed to these infectious agents. Eosinophil granule proteins have antiviral potential as marked by their ability to inhibit infectivity of respiratory syncytial virus, pneumonia virus of mice, rhinovirus, and influenza A virus (IAV). Antiviral functions that surpass the effects of granule proteins in eosinophils have also been delineated. We recently identified that eosinophils acted as putative antigen presenting cells during IAV infection, where virus exposure led to an upregulation of markers MHCI and CD86 and promoted cell migration and localization to the T cell zones of the draining lymph nodes. Virus infection in mice with heightened eosinophilia led to an elevated influx of IAV-specific CD8<sup>+</sup> T cells into the airways which may have protected the animals from severe influenza morbidity. Activated eosinophils were found in the bone marrow, spleen, mediastinal lymph nodes, and thymus of IAV-infected mice suggesting an active role for eosinophils during influenza. Eosinophils are susceptible to virus infection and reduce their oxygen consumption rate, temporally regulate activation (CD62L, CD69, PIR-A/B) and adhesion molecules (ICAM-1, VLA-4) in response to infection. IAV-induced cytopathology in respiratory epithelial cells was mitigated in the presence of eosinophils both *in vitro* and *in vivo*. Cumulatively, these data suggest a direct antiviral role for eosinophils through self-activation, reducing virus infectivity, and protecting the respiratory barrier from virus-induced damage further establishing an antiviral role for eosinophils in allergic airways.

# Invited Speaker Abstracts

## Eosinophil Phenotypes in Immune Pathways

Elizabeth Jacobsen

We and others have identified that eosinophils are immune modulating cells with multifunctional activities dependent in part upon differentiation into subtypes by their local microenvironment. The literature spans back decades regarding responses of eosinophils to stimulants, and the diverse cytokines and mediators they release. While these early studies were an entry into the eosinophil phenotype and subtype activities, renewed interest has developed in part due to discovering specific mediator release, rather than release of entire content, as well as finding new immune functions not only in asthma, but a wide variety of diseases and at homeostasis. For example, eosinophils are activated into subtypes by local cytokines, extracellular matrix, allograft responses, cancer, or infection leading to either immune suppression or activation of other cells. This session will review a sampling of the current state of eosinophil subtypes and the diversity of immune effector functions in health and disease. Moreover, we will describe our efforts to define cytokine induced type 2 and type 1 eosinophil subtypes using cytokines found to have an influence on eosinophil in vivo. Our preliminary data has shown type 2 eosinophils (E2 eosinophils) activated by IL-33 have been shown to induce effector T cell recruitment, M2 macrophage polarization, ILC2 activation, and type 2 inflammation in allergic models of asthma. Type 1 eosinophils (E1 eosinophils) exposed to INF- and TNF- have distinct effector functions such as production of type 1 chemokines (e.g., CXCL9) and upregulate unique cell surface molecules (e.g., PDL1). With our collaborators we show these eosinophils have unique activities in lung allograft to suppress T cell responses through PDL1 and iNOS dependent pathways. We anticipate this field will expand further and the characterization of eosinophil subtypes and their plasticity could be as complex as it is for macrophage, T cells, and innate lymphoid cells.

## Local Allergen Challenge Increases Tissue Eosinophil Populations at Remote Tissue Sites along the Skin:Lung:Gut Axis and Primes for Exacerbated Allergic Reactions to New Antigens

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The natural history of allergic diseases suggests a progressive relationship between allergic disorders of the skin, lung and gut, often referred to as the “atopic march”. Factors that drive the atopic march, including mechanisms that link remote allergen exposure and local allergic inflammatory responses between the skin, lung and gut are not yet known. Data emerging from our lab and several others are revealing phenotypically distinct subsets of tissue resident eosinophils, particularly within the intestinal tract and lung, and support the view that functions of tissue eosinophils are contextually defined. This study investigated the impact of remote allergen exposure on these phenotypically distinct tissue eosinophil populations. Our findings demonstrate that in allergen-sensitized mice direct allergen challenge of the skin, lung or gut elicited not only a local eosinophilic inflammation, but also increased the number and frequency of eosinophils within remote, allergen *non*-exposed lung and intestinal tissues. Remote allergen-elicited increase in lung eosinophils was associated with enhanced susceptibility to allergic airways inflammation induced by subsequent inhalation of a different allergen. These data add to our understanding of the distinct tissue subsets of eosinophils and provide mechanistic insights into the relationship of tissue eosinophils to the skin:lung:gut axis at the center of the atopic march.

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## Activated Eosinophils Exert Distinct Anti-Tumorigenic Activities in Colorectal Cancer

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Immunotherapies targeting T lymphocytes are revolutionizing cancer therapy yet they only benefit a subset of patients, especially in colorectal cancer (CRC). Thus, knowledge regarding additional cells in the tumor microenvironment (TME) is urgently required. Eosinophils are bone marrow-derived cells that have been largely studied in the context of allergic diseases and parasite infections. Despite the fact that tumor-associated eosinophilia has been described in various solid tumors including CRC, fundamental knowledge is still missing regard-



# Invited Speaker Abstracts

ing their activities and even the basic question of whether the TME promotes eosinophil recruitment without additional manipulation (e.g. immunotherapy) is unclear.

Herein, we report that eosinophils are swiftly recruited into developing tumors during induction of inflammation-induced CRC and in *Ap-c<sup>min/+</sup>* mice, which develop spontaneous intestinal adenomas. Using adoptive transfer and cytokine neutralization experiments, we demonstrate that the TME supported prolonged eosinophil survival independent of IL-5, a key eosinophil survival cytokine. Tumor-infiltrating eosinophils consisted of degranulating eosinophils and were essential for tumor rejection independently of CD8<sup>+</sup> T cells. Transcriptome and proteomic analysis revealed a functionally distinct IFN- $\gamma$ -linked signature for intratumoral eosinophils that was noticeably different from that of macrophages. Our data establish key anti-tumorigenic roles for eosinophils in CRC. These findings may facilitate the development of new pharmacological treatments unleashing anti-tumor responses by eosinophils especially in CRC patients displaying eosinophilia.

## Charcot-Leyden Crystals as a Drugable Target in Type 2 Immunity

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Although spontaneous protein crystallization is a rare event *in vivo*, Charcot-Leyden crystals (CLCs) consisting of galectin-10 (Gal10) protein are frequently observed in eosinophilic diseases, such as asthma. We found that CLCs derived from patients showed crystal packing and Gal10 structure identical to those of Gal10 crystals grown *in vitro*. When administered to the airways, crystalline Gal10 stimulated innate and adaptive immunity and acted as a type 2 adjuvant. By contrast, a soluble Gal10 mutein was inert. Antibodies directed against key epitopes of the CLC crystallization interface dissolved preexisting CLCs in patient-derived mucus within hours and reversed crystal-driven inflammation, goblet-cell metaplasia, immunoglobulin E synthesis, and bronchial hyperreactivity in a humanized mouse model of asthma. Thus, protein crystals may promote hallmark features of asthma and are targetable by crystal-dissolving antibodies

## “Siglec”ting the Allergic Response for Therapeutic Targeting

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Sialic acid-binding, immunoglobulin-like lectins (Siglecs) are single-pass transmembrane cell surface receptors found primarily on various leukocyte subsets. Most Siglecs, but not all, have conserved cytoplasmic signalling motifs that suggest they primarily function as inhibitory receptors. Among these, Siglec-8, first discovered in 2000, is expressed on human eosinophils, mast cells and weakly on basophils. Other Siglecs found on some or all of these same allergic effector cells as well as other cells include CD33, Siglec-6 and Siglec-7. Based on the work of several labs, it is now known that Siglec-8 engagement, either by specific antibodies or via multivalent, specific  $\alpha$ 2,3-linked sialylated, sulphated artificial or endogenous glycan ligands, can result in a number of responses *in vitro* including reduced eosinophil survival and altered integrin function, as well as reduced mast cell secretion responses, the latter also seen with agents that target CD33, Siglec-6 and Siglec-7 on mast cells and/or basophils. Its unique glycan ligand specificity has facilitated the discovery of endogenous tissue ligands for Siglec-8, including several identified so far from human upper and lower airways samples, and has allowed for the development of glycomimetic targeting molecules that permit nanoparticle attachment to Siglec-8 in a selective manner. Following Siglec-8 engagement, it gets internalized, which allows it to also function as an endocytic receptor for the delivery of therapeutic payloads. While Siglec-F is its closest paralog in the mouse, it is not a true ortholog and thus has its own unique pattern of expression, set of sialoside ligands and functions that often differ from Siglec-8. Because Siglec-8 is only expressed on human and primate cells, novel knock-in strains of mice have been developed in which Siglec-8 is expressed on eosinophils, mast cells, or both cell types, enabling further studies of its role *in vivo* and consequences of its targeting. Finally, a biotechnology company called Allakos, Inc. has created a humanized non-fucosylated IgG1 monoclonal antibody named AK002 that is being tested in clinical trials for the treatment of various diseases involving eosinophils and/or mast cells including eosinophilic gastrointestinal diseases, chronic urticaria, severe allergic conjunctivitis, and indolent systemic mastocytosis. To date, AK002 has demonstrated complete depletion of blood eosinophils within 1 hour of administration in

## Invited Speaker Abstracts

healthy volunteers. In mast cell diseases, AK002 has demonstrated symptomatic and quality of life improvements in open label studies of indolent systemic mastocytosis, and chronic spontaneous urticaria, including patients that are omalizumab (anti-IgE) naïve and omalizumab refractory, as well as inducible urticarias (cholinergic and dermatographism). Directly targeting eosinophils and mast cells via Siglec-8 represents a potential novel approach to treat allergic and inflammatory diseases.



# Oral Abstracts

## Real-Time In Vivo Imaging of Tissue Resident Eosinophils Under Homeostatic and Inflammatory Conditions

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Eosinophils are traditionally associated with allergic and parasitic inflammation.

More recently, eosinophils have also been shown to have roles in diverse processes including development, intestinal health, thymic selection, and B-cell survival. Despite this, tools to measure the dynamic activity of eosinophil *in situ* have been lacking. To better understand the role of tissue resident eosinophils, we used eosinophil-specific

CRE (eoCRE) mice to create GFP and tdTomato fluorescent reporter animals. We then used intravital microscopy to examine the dynamic behaviour of eosinophils in the healthy GI tract, mesentery, liver, lymph node, skin and lung. Robust numbers of eosinophils were found throughout the GI tract, and although the number of eosinophils was similar in these compartments, their morphology varied. At homeostasis, eosinophils were also found in the skin, liver, muscle, lymph node and lung. For example, we were able to directly observe eosinophils patrolling the lung vasculature at homeostasis. Given the role of eosinophils in allergic airway diseases, we also examined eosinophils in the lung following ovalbumin sensitization and challenge. In addition to counting eosinophils, we measured eosinophil behaviours including patrolling, crawling, clustering, tissue distribution and interactions with other leukocytes. Having these reporter mice allows eosinophils to be examined in real-time in living animals, paving the way to further understanding the roles eosinophils play in both health and disease.

This work was supported by a grant from the Canadian Institutes for Health Research.

## First Ultrastructural Demonstration of Galectin-10 in Resting and Activated Human Eosinophils

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**Background:** A predominant protein of human eosinophils is galectin-10 (Gal-10), also known as Charcot-Leyden crystal protein (CLC-P) because of its remarkable ability to cluster and form Charcot-Leyden crystals (CLCs), which are frequently found in tissues from patients with eosinophilic disorders. Attention has been paid to Gal-10 as a potential biomarker of eosinophil involvement in inflammation, but the subcellular localization of this protein within human eosinophils is still uncertain. In this work, we investigated the ultrastructural localization of Gal-10 in human eosinophils stimulated or not with inflammatory mediators as well as following cytolytic degranulation.

**Methods:** A pre-embedding immunogold transmission electron microscopy

(TEM) technique combined to other strategies for optimal cell preservation were used to investigate Gal-10 in eosinophils isolated from the peripheral blood of healthy donors and stimulated or not for one hour with tumor necrosis factor alpha (TNF- $\alpha$ ) or CC-chemokine ligand 11 CCL11 (eotaxin-1), recognized inducers of compound exocytosis and piecemeal degranulation (PMD), respectively. Additionally, cells were immunolabeled for Gal-10 after different times of cytolytic stimulation with phorbol-12-myristate 13-acetate (PMA) or immobilized IgG.

**Results:** First, a large amount of CLC-P/Gal-10 was clearly detected in the peripheral cytoplasm of resting eosinophils, a pattern also observed by immunofluorescence. Positivity was mostly seen below the plasma membrane (PM) with pools concentrated at specific PM domains. CLC-P/Gal-10 was also present on a vesicular pool and occasionally found scattered in the nucleus. Interestingly, labeling was not associated with specific granules. Quantitative analyses of different patients (n=5) and electron micrographs (n=150) showed the same pattern of peripheral distribution of CLC-P/Gal-10 with negative secretory granules in resting eosinophils. Second, TNF- $\alpha$  and CCL11 stimulation did not change this immunolabeling pattern, indicating that secretion of CLC/Gal-10 is not connected with compound exocytosis or PMD. On the other hand, cytolysis induced an extraordinary secretion and crystallization of CLC/Gal-10. Cells drastically changed their intracellular localization of CLC-P/Gal-10 and showed high number of PM-derived extracellular vesicles (EVs) accumulating CLC-P/Gal-10. Crystallization occurred in association with EVs, large vacuoles and free cytoplasmic pools after PM disruption. CLCs of different sizes (growing CLCs) were seen extracellularly among CLC/Gal-10-negative free-extracellular granules.

**Conclusions:** Our data provide direct evidence that CLC/Gal-10 is stored within human eosinophils in the peripheral cytoplasm, keeping a close link with the PM and vesicular pools but not with secretory granules. Secretion and crystallization of Gal-10 rely on cytolytic degran-

# Oral Abstracts

ulation and formation of EVs but are not associated with secretory pathways of PMD and compound exocytosis. Our findings shed light on how human eosinophils store and release their constitutive pools of Gal-10 during inflammatory responses.

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## SIGLEC-8 Signals through an Unanticipated Set of Molecules to Induce Integrin Upregulation and Activation, ROS Production, and Cell Death in Eosinophils

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**Background:** The receptor known as sialic acid-binding immunoglobulin-like lectin (Siglec)-8 is selectively expressed on eosinophils, mast cells, and basophils. Our previous work has shown that engagement of Siglec-8 on IL-5-primed eosinophils causes cell death via activation of the  $\beta_2$ -integrin Mac-1 and NADPH oxidase activity that a subset of signaling molecules including PI3K and Rac-1 is involved in this process, and that cytoskeletal reorganization occurs in response to Siglec-8 signaling. However, the identities of other signaling molecules responsible for the induction of cell death and whether these and other mediators act upstream or downstream of integrin involvement remain unknown.

**Methods:** Using the monoclonal antibody 2C4 against Siglec-8 and pharmacological inhibition in combination with assays to measure integrin cell surface upregulation, integrin activation, ROS production, and cell death in IL-5-primed human eosinophils, we sought to identify additional mediators involved in Siglec-8 signaling and assess where in the pathway these molecules play a role.

**Results:** We now demonstrate that the enzymatic activities of Syk, SHIP-1, RIPK3, MEK1, ERK1/2, and Btk are also necessary for Siglec-8-induced eosinophil cell death and act upstream of ROS generation. While most of these molecules are necessary for Siglec-8-induced upregulation of CD11b (Mac-1 integrin subunit  $\alpha_M$ ) at the eosinophil cell surface, Btk is phosphorylated and activated late in the signaling cascade and is necessary for CD11b activation and ROS production but not CD11b upregulation. In addition, actin cytoskeletal disruption using latrunculin B or jasplakinolide prevents Siglec-8 engagement-induced eosinophil death, and the results demonstrate that actin depolymerization is necessary for surface integrin upregulation and that actin polymerization is necessary for integrin engagement-induced ROS production in eosinophils.

**Conclusions:** These results show that Siglec-8 signals through an unexpected and unique set of cytoplasmic molecules in IL-5-primed eosinophils to induce adhesion- and ROS-dependent cell death that in some but not all ways resembles necroptosis. These findings challenge the dogma that siglecs with ITIM or ITIM-like cytoplasmic motifs signal through inhibitory pathways involving SHP1 or SHP2 to achieve their downstream functions.

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## Identification of Eosinophil-Specific Gene-Enhancer Regions

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**Background:** Expression of eosinophil-specific genes is one of the driving forces directing eosinophil development, tissue specificity and function. The epigenetic mechanisms that facilitate gene transcription are relatively unknown in the context of eosinophil maturation. Use of bioinformatic techniques to identify gene enhancers and promoter regions has played an important role in delineating regulation of macrophage and lymphocyte development and function, however little has been done to utilize these techniques in the field of eosinophil biology. Application of these techniques will provide a much needed insight to genes that regulate eosinophil development, subset and function, meeting a currently unmet need for the eosinophil research community.

**Methods:** ATAC-seq and ChIP-seq were performed with eosinophils cultured from whole bone marrow cells and with native eosinophils to identify transcriptionally active regions of DNA and active enhancer marks, respectively, to identify eosinophil subset-specific regulatory elements.

**Results:** An eosinophil-specific enhancer element in the gene *Il5ra*, which encodes the receptor for the eosinophil-promoting cytokine IL-5 was identified. ATAC-seq revealed an open region of chromatin in intron 10 of the mouse *Il5ra* gene between positions chr6:106,725,541→106,726,394, located close to the alternative splice site for generation of the two soluble IL-5Ra isoforms. ChIP-seq analysis of this region confirmed the presence of histone modifications associated with active enhancer marks (H3K27ac, H3K4me3) in eosinophils and EoPs,

## Oral Abstracts

which were absent in cells not of the eosinophil lineage (e.g. macrophages). Further analysis revealed >67,300 unique non-promoter eosinophil-specific regulatory elements with active enhancer marks contained inside open areas of chromatin, suggesting numerous more regulatory elements are present in the genome with currently unknown functional effects.

**Conclusions:** These data provide proof of principle for the validity and feasibility of applying bioinformatics approaches in the field of eosinophil biology, allowing identification of the currently unidentified gene regulatory elements which are essential for eosinophil development, tissue specificity and function. Identification of these gene regulatory elements will allow future studies involving deletion of candidate enhancer elements using CRISPR to investigate the function of these genetic elements.

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### Single Cell RNA Sequencing of Inflammatory Tissue T-Cells in Eosinophilic Esophagitis

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**Background:** Heterogeneity of T cells is an innate feature of the mammalian adaptive immune system and underlies protective immunity and inflammatory responses. We aim to resolve the heterogeneity of human tissue CD3<sup>+</sup> T cells during allergic inflammation, focusing on a tissue-specific allergic disease, eosinophilic esophagitis (EoE) at the single cell levels.

**Methods:** We applied single-cell RNA sequencing (scRNA-seq) to profile 1088 human T cells residing in the tissues of subjects with healthy and allergic states to reveal the diversity of transcriptional phenotypes that can be adopted by tissue T cells upon allergic inflammation. We used an unsupervised approach to define tissue T cell subtypes was performed by principal components analysis (PCA) to screen for those genes whose expression most contributed to the observed variations among different subgroups.

**Results:** Eight disparate tissue T cell subtypes (designated T1-T8) were identified, with T7 and T8 enriched in the diseased tissue. The phenotypes of T7 and T8 resemble putative T<sub>REG</sub> (FOXP3<sup>+</sup>) and effector Th2 (GATA3<sup>+</sup>)-like cells, respectively. Prodigious levels of IL-5 and IL-13 were confined to HPGDS<sup>+</sup> CRTH2<sup>+</sup> IL-17RB<sup>+</sup> FFAR3<sup>+</sup>CD4<sup>+</sup> T8 effector Th2 cells. EoE severity closely paralleled a lipid/fatty acid-induced activation node highlighted by the expression of the short-chain fatty acid receptor FFAR3. Ligands for FFAR3 induced Th2 cytokine production from human and murine T cells including in an in vivo allergy model.

**Conclusion:** We have elucidated the defining characteristics of tissue-residing CD3<sup>+</sup> T cells in EoE, a specific enrichment of CD4<sup>+</sup> T<sub>REG</sub> and effector Th2 cells, confinement of type 2 cytokine production to the CD4<sup>+</sup> effector population, a highly likely role for FFAR3 in amplifying local Th2 responses in EoE, and a resource to further dissect tissue lymphocytes and allergic responses.

**Grant Support:** This work was supported by the HOPE Research Award by American Partnership for Eosinophilic Disorders (APFED), CCHMC's Digestive Health Center Pilot & Feasibility Research Award (P30 DK078392), and CCHMC's Trustee Award. The NGS expenses were partially subsidized by P30 DK078392. This work was supported in part by NIH U19 AI070235, NIH R01 AI124355, R37 R37 A1045898, the Campaign Urging Research for Eosinophilic Disease (CURED) Foundation, and the Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning.

### Characterization of a Mouse Model of Heart Disease in Hypereosinophilia

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Hypereosinophilic syndrome is characterized by sustained blood and/or tissue hypereosinophilia leading to tissue damage and organ dysfunction. Morbidity and mortality occur primarily due to cardiac and thromboembolic complications. Understanding the cause and mechanism of disease would aid in the development of targeted therapies with greater efficacy and fewer side effects.

We discovered a spontaneous mouse mutant in our colony with a hypereosinophilic phenotype. Mice develop peripheral blood eosinophilia, infiltration of lungs, spleen and heart by eosinophils, and extensive myocardial damage and remodeling. This ultimately leads to heart failure and premature death, often by 15 weeks of age. Histopathologic assessment of the hearts from affected mice revealed a robust inflammatory infiltrate of the ventricular myocardium. This infiltrate is composed primarily of eosinophils and B-lymphocytes, leading to myocardial damage and replacement fibrosis, consistent with eosinophilic myocarditis (EM). In many cases, hearts showed dilatation and

## Oral Abstracts

thinning of the right ventricular wall, suggestive of an inflammatory dilated cardiomyopathy. Most mice examined primarily showed atrial thrombi, which often filled the entire/majority of the chamber. RNA and protein expression analysis revealed overexpression of genes and proteins involved in adaptive (humoral, Th1 and Th2) immunity, innate immunity and inflammatory responses. Disease could be transferred to wild type mice by adoptive transfer of splenocytes from affected mice, suggesting a role for the immune system in the observed phenotype.

The pathologies observed in the mutant line are reminiscent of those seen in patients with hypereosinophilia, where cardiac-related morbidities, like congestive heart failure and thrombi, are the most common causes of death. As such, our model provides an opportunity to test mechanistic hypotheses and develop targeted therapies.

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### Asthma is Associated with Brain Neurodegeneration: A Possible Effect of Eosinophilic Inflammation?

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**Background:** Asthma is associated with a significant increase in the risk for cognitive impairment. Moreover, the development of dementia and Alzheimer's disease is increased in asthma and parallels the frequency of exacerbations raising possible links between peripheral inflammation, i.e. as in asthma, and risks for neurodegeneration of the brain. Our previous work has used an inhaled allergen challenge to demonstrate that the resulting airway inflammation is associated with an activation of brain neurocircuits, which responses correlate with increases in sputum eosinophils and falls in lung function. The goal of following study was to determine if chronic asthma is also associated with changes in the brain white matter representing neurodegeneration. We hypothesized that asthma subjects, particularly those with severe disease and greater markers of inflammation, will have poorer white matter integrity and evidence of neurodegeneration. Moreover, these white matter changes will be associated with greater asthma severity and poorer cognitive function.

**Methods:** In our investigations, 111 participants with asthma and 135 healthy controls made up the study population. The asthma subjects varied in severity and included participants with mild (n=19), mild-to-moderate (n=67) and severe (n=25) disease. These results were compared to normal controls (n=135). Principle component analysis was used to define severity for each subject and was based on medication use, symptoms, and lung function. Asthma lifetime burden was represented by the asthma severity score x number of years since asthma diagnosis. Diffusion tensor imaging (DTI) and Neurite orientation and dispersion density imaging (NODDI) of the brain were used to measure the integrity of white matter microstructure, changes of which serve as markers of neurodegeneration. In addition, an algorithm was applied to these structural brain images that estimates brain age, based on a very large normative dataset, to determine if characteristics of asthma relate to accelerated brain aging.

**Results:** Compared to the normal controls, asthma subjects had significant changes in neurite density, mean diffusivity and free water fraction all representing deleterious alterations in white matter, such as axonal loss. These changes were proportional to the severity of existing asthma. Cognitive testing in subjects with severe asthma revealed worse function in those with a greater lifetime burden of asthma, after controlling for age. Finally, we found a greater asthma lifetime burden and peripheral blood eosinophils to be associated with accelerated brain aging, which was associated with poorer cognitive function.

**Conclusions:** Our observations of brain structure suggest that neurodegenerative changes are found in asthma, and may reflect an increased risk factor for dementia. Although the mechanisms underlying the degenerative changes in asthma are not established, we speculate a possible role for eosinophils in these deleterious effects.

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### The Role of Adipose Tissue-Resident Eosinophils in Adipocyte Metabolism and Whole-Body Energy Homeostasis

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**Background:** Adipose tissue resident eosinophils (AT-EOS) are thought to play a pivotal role in whole-body energy homeostasis. However, the precise role of, and the mechanisms underlying AT-EOS in the regulation of whole-body energy metabolism remains elusive. This is because of the limitations of rodent models which have been genetically engineered to either produce elevated whole-body eosinophils or knock-out models for this hematopoietic lineage.

## Oral Abstracts

**Methods:** To determine the function of AT-EOS, we generated a transgenic mouse model overexpressing human eotaxin 2 (hEo2), which is an eosinophil specific chemokine, under the control of a fat-specific  $\alpha$ P2 promoter, to recruit circulating EOS exclusively into adipose (hEo2tg).

**Results:** Compared to wild type (WT) littermates of both genders, hEo2tg mice displayed a significant increase of AT-EOS in multiple AT depots including, perirenal and subcutaneous white adipose tissue (sWAT). However, EOS were not increased in brown adipose tissue (BAT). When fed a high-fat diet (HFD), hEo2tg mice gained less weight (Males weight:  $45.5 \pm 0.7$ g vs  $51 \pm 0.4$ g,  $P < 0.01$  hEo2tg vs WT) and adipose tissue (Males %fat:  $20.8 \pm 0.2$  vs  $31.7 \pm 0.6$ ,  $P < 0.01$  hEo2tg vs WT) compared to WT. Transgenic mice showed improved glucose tolerance (GTT) compared to WT (Males GTT AUC:  $300054 \pm 121$  vs  $320865 \pm 920$ , hEo2tg vs WT  $P < 0.001$ ). This was accompanied by increased energy expenditure and heat production compared to WT. These phenotypes were equally found in female animals. Moreover, the adipocytes in hEo2tg were smaller in both genders, and the expression of genes involved in fat oxidation in BAT and sWAT was upregulated in hEo2tg mice compared to WT mice.

**Conclusions:** These data suggest that WAT-resident EOS contribute to increased BAT activation, browning of sWAT and to whole-body energy expenditure. Additional studies will be needed to evaluate the molecular mechanism(s) underlying the benefits of AT-EOS.

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### Eosinophils in Beige Adipose Tissue Activation

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Obesity is a global problem and represents a significant health and economic burden. Recently a new category of brown fat-like cells, "beige" cells residing within white fat, have been identified. These cells burn fuels to generate heat and therefore may reduce obesity by burning rather than storing excess energy. Cells of the immune system – macrophages, innate lymphoid cells and eosinophils – appear to be essential in the "beiging" of adipocytes.

Mice with a deletion in the gene encoding the transcription factor Kruppel-like Factor 3 (KLF3) are lean and are protected from diet-induced obesity. Interestingly, these mice show evidence of more beige fat and an increased capacity for thermogenesis. The adipocytes were not responsible for this phenomenon so to test the involvement of adipose-resident immune cells, we performed a bone marrow transplantation and were able to confer the lean beige phenotype on otherwise wild type mice. This suggested that KLF3 deficiency in haematopoietic lineages drives leanness in this mouse model. We interrogated different types of adipose-resident immune cells and have focused on eosinophils in this mouse model, partly because of the high expression of KLF3 in human and mouse eosinophils.

We performed what we believe to be the first genome-wide expression analyses on eosinophils isolated from white adipose tissue and uncovered widespread gene expression differences in the absence of KLF3. This suggests that KLF3 is an important regulator of gene expression within eosinophils from this tissue niche. Interestingly, we saw expression of a number of genes that encode secreted proteins known for their role in beiging. We have validated these as direct KLF3 target genes using chromatin immunoprecipitation in EoL-1 cells to demonstrate that KLF3 directly binds to regulatory regions of these genes. Our data suggest that eosinophils may contribute to beige fat activation by secreting these factors. We also detected expression of a number of novel secreted proteins in adipose tissue-derived eosinophils. We are now testing whether these novel secreted proteins, which we term "eosinokines" are able to induce beiging and energy expenditure in cell culture and *in vivo* models, which may lead to new therapeutic agents to drive beiging and combat obesity.

**Grant Support:** AK was supported by an Australian Postgraduate Award. EV is supported by a Scientia Scholarship. KQ is supported by a Scientia Fellowship.

### Idiopathic Eosinophilic Vasculitis, Another Side of Hypereosinophilic Syndrome? A Comprehensive Analysis of 115 Cases in Asthma-Free and ANCA-Negative Patients

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# Oral Abstracts

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**Background:** In a previous work in which we compared the clinical and biological characteristics of hypereosinophilic syndrome (HES) and eosinophilic granulomatosis with polyangiitis (EGPA), we reported on vasculitic manifestations occurring in asthma-free HES patients with negative ANCA serology. According to both the 2012 Chapel Hill Conference Consensus (CHCC2012) and the European Respiratory Society-endorsed taskforce on EGPA, the absence of asthma rules out EGPA diagnosis. Hence, we aimed in the present study to review clinical, biological and histopathological features of asthma-free and ANCA-negative patients with eosinophilic vasculitis (EoV), and to further suggest provisional classification criteria of this entity.

**Methods:** We conducted a systematic review of our hospital database and of literature in order to collect cases of asthma-free HES patients who fulfilled all 4 following criteria: (1) histopathological or clinical features of eosinophilic vasculitis (biopsy-proven vasculitis with predominant eosinophilic infiltration of the vessel wall and/or clinical surrogates of vasculitic features with blood hypereosinophilia > 1.5G/L), (2) absence of other disorders or conditions as major reason for organ damage, including any other vasculitis defined in CHCC2012, (3) absence of ANCA, and (4) absence of persistent/active asthma.

**Results:** Ten of our 83 (12%) asthma-free HES patients and 105 additional cases retrieved in the literature fulfilled such criteria for EoV, among which 43 were considered to have a single-organ EoV (including isolated coronary EoV, n=31, temporal EoV, n=8, and cerebral EoV, n=4), and 72 to have an EoV. Among EoV patients, the recurrent clinical manifestations were cutaneous vasculitis (53%), neuropathy (24%), thromboangiitis obliterans-like disease (15%), deep venous thrombosis (11%), lung involvement (11%), cerebral vasculitis (10%), ear-nose-throat manifestations (8%), digestive manifestations (8%), coronary arteritis (7%), liver and/or spleen involvement (7%), eosinophilic fasciitis (6%), arthralgia and/or myalgia (6%), temporal arteritis (4%) and inflammatory ocular involvement (4%). In addition, at least 15 patients (21%) developed biopsy-proven (non vasculitic) HES manifestations. Blood hypereosinophilia >1.5 G/L was present in 87% (n=55/63). Necrotizing vasculitis was found in 28 of 60 (47%) available biopsies. Granuloma was only found in n=7/60 (12%), including 2 patients who fulfilled the 1990 American College of Rheumatology criteria for EGPA despite the absence of asthma. According to histopathological and/or radiological features, EoV involved small-sized vessels in 59 cases (82%), medium sized vessels in 17 cases (24%) and/or large sized vessels in 14 cases (19%). In addition to vessel necrosis, we report some potential mechanisms of vessel damaging induced by eosinophils: extracellular deposition of eosinophil granule proteins (n=8/72 EoV) suggest activated eosinophils, but they may have also contributed to arterial aneurysms (n=6/115, 5%), occlusive intimal fibrosis (n=12/115, 10%), and/or focal thrombosis (n=23/115, 20%).

**Conclusions:** This comprehensive literature review highlights that multiple cases of asthma-free and ANCA-negative patients fulfilling classification criteria for EoV have previously been reported. Hence, it seems advisable that, in the context of blood hypereosinophilia, the advent of vasculitic manifestations (either organ-limited or systemic) should be considered as HES-defining features. Last, updated classification criteria for vasculitis should consider EoV, or *HES-related vasculitis*, as an additional cause of "variable-vessel size" vasculitis.

## Lung Eosinophils Increase Vagus Nerve Mediated Airway Reflex Bronchoconstriction in Mice

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**Background:** Eosinophils can mediate airway hyperresponsiveness by increasing vagally mediated reflex bronchoconstriction. Here we test the role of circulating and airway eosinophils in changing airway nerve function.

**Methods:** Airway resistance in response to aerosolized 5-hydroxytryptamine (5-HT, 10-300 mM) was measured in mice with elevated eosinophils in blood only (NJ1638), in blood and airway (NJ1726), and with high IL-5 but no eosinophils (NJ1726 PHIL). The effects of vagotomy and of atropine were used to discriminate the contribution of the nerves vs. airway smooth muscle to reflex bronchoconstriction. Inflammatory cells in bronchoalveolar lavage (BAL), blood, and bone marrow were also quantified.

**Results:** Blood eosinophils were increased in both NJ1638 and NJ1726 mice compared with wild-type. In contrast, only NJ1638 mice had increased eosinophils in bone marrow while only mice had increased eosinophils in BAL. When vagi were intact, airway resistance in response to aerosolized 5-HT was significantly increased only in NJ1726 mice. Vagotomy or atropine each blocked 5-HT induced bronchoconstriction, demonstrating that it was mediated via vagal reflex. This response was not increased in NJ1738/PHIL or NJ1638 mice, demonstrating that it required lung eosinophils, but not blood eosinophils or IL5 in the absence of eosinophils. Airway responses to methacholine were not different among strains of mice, indicating eosinophils did not change M3 function on airway smooth muscle.

# Oral Abstracts

**Conclusions:** Lung eosinophils, not circulating eosinophils or IL5, increase vagally mediated reflex bronchoconstriction. Therapeutic interventions that block or reduce lung eosinophils may effectively inhibit reflex induced airway hyperreactivity mediated by vagus nerves in eosinophilic asthma.

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## Efficacy of Rpc4046, an Anti-Interleukin-13 Monoclonal Antibody, in Patients with Active Eosinophilic Esophagitis: Analysis of the Steroid-Refractory Subgroup from the Heroes Study

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**Background:** The HEROES study (NCT02098473) was a 16-week, double-blind, placebo-controlled, phase 2, multicenter study that evaluated the efficacy and safety of RPC4046 in adult patients with active eosinophilic esophagitis (EoE). The study demonstrated RPC4046 treatment significantly reduced esophageal eosinophil count, improved histopathologic parameters, and improved patient perception of disease severity and symptoms. The HEROES study included subjects who were considered steroid refractory based on prior corticosteroid use and investigator judgment. Both prespecified and post-hoc analysis were undertaken to assess the effect of RPC4046 treatment on this important subgroup.

**Methods:** In this study, 99 adult patients with active EoE were stratified by steroid refractory status (yes/no) and randomized 1:1:1 to receive RPC4046 180 mg, 360 mg, or placebo weekly for 16 weeks. The primary endpoint was change from baseline in mean esophageal eosinophil count at week 16. Secondary endpoints included mean change from baseline to week 16 in EoE Endoscopic Reference Score (EREFS), improvements in dysphagia determined by the Daily Symptom Diary (DSD), Eosinophilic Esophagitis Symptom Activity Index (EEsAI) score, and EoE Histology Scoring System (EoEHSS) based on grade and stage.

**Results:** Of the steroid-refractory patients enrolled, 16 were randomized to placebo, 14 to RPC4046 180 mg, and 17 to RPC4046 360 mg. The differences in change in mean esophageal eosinophil counts from baseline to week 16 between RPC4046 180 mg and placebo as well as between RPC4046 360 mg and placebo were statistically significant ( $P \leq 0.0001$  for both comparisons; Table). The difference in mean change in EREFS (total) between each RPC4046 group and the placebo group was statistically significant for total score over all esophageal locations ( $P < 0.01$  for both comparisons). The mean change in DSD composite score in the RPC4046 360 mg group compared with the placebo group approached statistical significance ( $P = 0.0547$ ). Statistically significant improvements from baseline to week 16 in the RPC4046 treatment groups were also observed on histology as determined by EoEHSS ( $P < 0.0001$  for each RPC4046 group vs placebo on both grade and stage scores) and on symptom severity as determined by EEsAI ( $P = 0.0852$  and  $P = 0.0393$  for the RPC4046 180 mg and 360 mg groups vs placebo, respectively).

**Conclusions:** RPC4046 treatment improved mean and peak eosinophil count and histopathologic parameters, improved endoscopic features, and improved symptoms in steroid-refractory EoE patients. Although this analysis was undertaken in a subgroup of patients in the HEROES study, these data provide support that treatment with RPC4046 results in marked improvement in multiple EoE-related disease measures in steroid-refractory EoE patients.

| Esophageal Eosinophil Counts, EREFS, and DSD Score in the Steroid-Refractory Subgroup |                   |                           |                           |
|---|-------------------|---------------------------|---------------------------|
|   | Placebo<br>(n=16) | RPC4046 180 mg<br>(n=14)  | RPC4046 360 mg<br>(n=17)  |
| <b>Esophageal Eosinophil Count (eosinophils/hpf)</b>                                  |                   |                           |                           |
| Baseline mean (SD)  | 79.2 (47.1)       | 146.9 (83.1)              | 127.5 (78.2)              |
| Week 16 mean (SD)   | 101.6 (64.7)      | 27.5 (35.4)<br>$P=0.0001$ | 28.3 (34.8)<br>$P<0.0001$ |
| Week 16 number (proportion) <15 peak  | 0/15              | 5/12 (0.42)<br>$P=0.0066$ | 8/15 (0.53)<br>$P=0.0012$ |
| <b>EREFS (Total)</b>  |                   |                           |                           |
| Baseline mean (SD)  | 10.7 (3.8)        | 9.9 (4.8)                 | 9.7 (3.9)                 |
| Week 16 mean (SD)   | 10.4 (4.7)        | 5.8 (4.9)<br>$P=0.0026$   | 5.3 (3.7)<br>$P=0.0016$   |



# Oral Abstracts

| DSD Composite Score |             |                                 |                                 |
|---------------------|-------------|---------------------------------|---------------------------------|
| Baseline mean (SD)  | 32.7 (12.0) | 26.9 (14.6)                     | 29.3 (9.4)                      |
| Week 16 mean (SD)   | 31.0 (18.1) | 25.6 (17.8)<br><i>P</i> =0.8284 | 16.9 (19.0)<br><i>P</i> =0.0547 |

DSD, Daily Symptom Diary; EREFS, EoE Endoscopic Reference Score; hpf, high-powered field; SD, standard deviation.

## Functional Role of Kallikrein 5 and Proteinase Activated Receptor (PAR) 2 in Eosinophilic Esophagitis

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**Background:** Eosinophilic esophagitis (EoE) is a chronic, food antigen driven, inflammatory disease of the esophagus associated with impaired barrier function. Evidence is emerging that loss of esophageal expression of the serine peptidase inhibitor, kazal type 7 (SPINK7) is an upstream event in EoE pathogenesis. The mechanism by which SPINK7 mediates its function is by mostly unknown.

**Methods:** A functional screen of candidate proteases was performed in attempt to find direct targets of SPINK7. KLK5 expression was manipulated by overexpressing KLK5 in an esophageal epithelial cells (EPC2 cells) and by CRISPR/Cas9 gene deletion of KLK5 in mice. Epithelial cells were differentiated and assessed by several experimental approaches including FITC-dextran flux, epithelial resistance measurements, proteolytic activity, and cytokine release. An experimental EoE murine model was induced by egg ovalbumin and esophageal proteolytic activity was assessed by fluorescent substrates. Serum IgE, mast cell protease 1 (MCPT1) levels and the number of eosinophils in the esophagus, lungs and blood were analyzed in *Klk5*<sup>-/-</sup> mice compared to *Klk5*<sup>+/+</sup> mice. To interfere with a KLK5 downstream pathway, PAR2 was blocked using a PAR2 selective antagonist ENMD-1068 in epithelial cells in vitro, ex vivo and in vivo in an experimental EoE murine model. KLK5 activity was inhibited in vivo by delivering A1AT to the esophagus. A1AT was conjugated to a fluorescent molecule and its delivery, stability and functionality in the esophagus were analyzed. The effect of A1AT delivery in an experimental EoE murine model was assessed.

**Results:** In vitro functional assays of SPINK7 revealed that SPINK7 is a direct inhibitor of KLK5 (*K<sub>i</sub>* = 130 nM). Overexpression of *KLK5* in differentiated esophageal epithelial cells recapitulated the effect of *SPINK7* gene silencing including barrier impairment and pro-inflammatory cytokine release. Furthermore, deficiency of KLK5 in vivo (*Klk5*<sup>-/-</sup> mice) decreased allergen-induced esophageal proteolytic activity and eosinophilia. The KLK5 substrate, PAR2, was elevated in the esophagus of human EoE compared to control individuals and a PAR2 antagonist inhibited cytokine production associated with loss of SPINK7 in human epithelial cells in vitro. Furthermore, in-vivo inhibition of PAR2 and delivery of a non-specific serine protease inhibitor,  $\alpha$ 1 anti-trypsin (A1AT), attenuated a murine model of allergen-induced esophageal eosinophilia.

**Conclusions:** We have provided evidence that loss of *SPINK7* mediates its pro-EoE effects via kallikrein (KLK) 5 and protease-activated receptor 2 (PAR2), at least in part. These findings demonstrate a role for the balance between KLK5 and protease inhibitors in the esophagus and highlight EoE as a protease-mediated disease. As such, antagonizing KLK5 and/or PAR2 has potential to be therapeutic for EoE.

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## Activated Eosinophil Subsets Are an Integral Part of the Tumor Microenvironment in Lung Metastasis, Displaying Anti-Tumorigenic Activities

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**Background:** Cancer-associated mortality is nearly exclusively a result of tumor metastasis, and the lung is one of the main metastatic sites for multiple tumors. Mucosal surfaces such as the lungs are a natural homeostatic niche for eosinophils under baseline conditions,

## Oral Abstracts

and in many inflammatory conditions. Furthermore, key functions have been identified for “tissue-resident” and “recruited” eosinophils in the lungs. Therefore, we were set to characterize the phenotypes and roles of eosinophils in lung metastasis.

**Methods:** Experimental lung metastasis was induced by tail vein injections of PyMT (breast cancer), B16-F10 (malignant melanoma) and MC38 (colorectal cancer) cells into wild type and  $\Delta$ dblGATA mice. Infiltration of eosinophils was determined by flow cytometry and immunohistochemistry. Tumor burden was assessed by continuous bioluminescent *in-vivo* imaging and manual counting of tumor nodules. The ability of tumor-secreted factors to induce eosinophil accumulation was determined following five daily intraperitoneal injections of tumor cell conditioned media. For *in vitro* studies, eosinophils were purified from the peritoneum of *Il5Tg* mice or from peripheral blood of healthy donors and cultured with conditioned media of various mouse and human tumor cell lines.

**Results:** Intravenous injections of PyMT, B16-F10 and MC38 cells resulted in formation of tumor nodules in the lungs, which was associated with a 5-fold increase in eosinophil recruitment. Recruited eosinophils were activated as measured by the increased Siglec-F and CD125 expression. At least two eosinophil subsets were identified within the tumor microenvironment (TME) following PyMT, B16-F10 and MC38 cell injections as defined by the expression of Siglec-F, CD125 and CD101. Anti-MBP immunohistochemical staining revealed that eosinophils reside in distinct anatomical compartments in the metastatic lung (i.e., the lung parenchyma and within tumor nodules). Consistent with our *in vivo* findings, tumor-secreted factors, which were present in culture media of PyMT, B16-F10 and MC38 cells, induced rapid and marked eosinophil migration *in vitro* and following intraperitoneal injections *in vivo*. In addition, tumor-secreted factors prolonged eosinophil survival. Similarly, conditioned media of human colorectal, melanoma and breast cancer cells, prolonged human eosinophil survival *in vitro* as well. Intravenous injections of PyMT and B16-F10 cells resulted in markedly increased tumor burden in  $\Delta$ dblGATA mice in comparison with wild type mice. In support of these findings, co-culture of eosinophils with PyMT cells (E:T ratio 1:5), resulted in significant eosinophil-driven cytotoxicity towards the tumor cells. Interestingly, the *in vivo* antitumor activities of eosinophils towards PyMT cells were lung-specific since no difference was observed between wild type and  $\Delta$ dblGATA mice, in a model of primary breast cancer by orthotopic PyMT injections.

**Conclusions:** Our data demonstrate that eosinophil recruitment and antitumor activities towards metastatic cells in the lung, occurs in response to several tumor cell types. Furthermore, our data suggests that the antitumor activities of eosinophils are dependent at least in part by local factors secreted by the tumor cells in the lungs. Thus, eosinophils could serve as a novel cellular target for immunotherapy in lung metastasis.

### Eosinophils Promote GM-CSF-Dependent Anti-Tumor Immunity Through Activation of Type I T-Cell Responses

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**Background:** Eosinophils are predominantly known for their pathogenic roles in allergic diseases and the depletion of eosinophils represents an efficient strategy to alleviate allergic asthma. The consequences of eosinophil deficiency for human health remain poorly understood.

**Methods:** To elucidate a possible role of eosinophils in cancer, we have combined syngeneic models of colorectal cancer (CRC) with mouse strains that lack critical signaling pathways in eosinophils. We have further generated a tissue microarray comprising 240 CRC resection specimens and have used it to examine the prognostic value of eosinophil infiltration.

**Results:** The genetic loss of eosinophils severely compromises anti-tumor immunity, which can be attributed to defective Th1 and CD8+ T-cell responses. The specific loss of GM-CSF signaling or of IRF5 expression in the eosinophil compartment phenocopies the loss of the entire lineage. GM-CSF activates IRF5 *in vitro* and can be administered recombinantly to improve tumor immunity. IL-10 counter-regulates IRF5 activation by GM-CSF. Consequently, the eosinophil-specific loss of IL-10 signaling, or the excessive production of eosinophils, both result in improved tumor control. Neutralization of TNF $\alpha$  and the deletion of the TNF- $\alpha$  receptor in tumor cells abrogate tumor control. CRC patients whose tumors are infiltrated by large numbers of eosinophils also exhibit robust CD8 T-cell infiltrates and have a better prognosis than patients with eosinophil-low tumors.

**Conclusions:** Our study demonstrates a critical role of eosinophils in tumor control in CRC and introduces the GM-CSF/IRF5 axis as a critical driver of the anti-tumor activities of this versatile cell type.

# Oral Abstracts

## Alphaiib Integrin (cd41) on Blood Eosinophils is a Potential Biomarker for Disease Activity in Eosinophilic Esophagitis (eoe)

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**Background:** Monitoring of eosinophilic esophagitis (EoE) would benefit from a biomarker to replace invasive endoscopic procedures and pathology. We hypothesized that blood eosinophil surface marker(s) related to eosinophil activation are associated with the peak eosinophil count (PEC) on esophageal biopsy in EoE; specifically, beta1 integrin activation, which enables arrest of eosinophils in inflamed vessels and predicts disease activity in non-severe asthma; P-selectin, which activates beta1 integrin; and alphallb integrin, a reporter of platelet association with eosinophils.

**Methods:** Twenty-five EoE patients were recruited following two-month proton-pump-inhibitor therapy and diagnostic endoscopy, with visit 1 (V1) approximately one week after endoscopy. Patients received standard of care EoE treatment (swallowed steroid or food elimination) for two months followed by visit 2 and repeat endoscopy (V2). Beta1 integrin activation (mAb N29 intensity), eosinophil-surface P-selectin (CD62P) and alphallb integrin (CD41), along with 13 other eosinophil-surface markers were assayed by whole blood flow cytometry. The PEC per high power field (HPF) was assessed on esophageal biopsy. Receiver operating characteristic (ROC) curve analysis for the ability of N29, P-selectin, and alphallb to predict PEC was performed.

**Results:** There were significant correlations among N29, P-selectin, and alphallb integrin at V1 or V2. One or more of the three measurements, N29 reactivity or P-selectin or alphallb-positive blood eosinophils, decreased from V1 to V2 in 19 of the 25 patients. ROC analysis demonstrated that the percentage of alphallb-positive blood eosinophils at V2 predicted a PEC < 6/HPF with an area under curve (AUC) = 0.84 ( $p = 0.004$ ) and < 15/HPF with AUC = 0.79 ( $p = 0.02$ ). An optimal cutoff criterion of < 22.9% alphallb-positive blood eosinophils predicted PEC < 6/HPF with sensitivity = 82% and specificity = 93%, whereas a criterion of < 26.7% alphallb-positive blood eosinophils predicted PEC < 15/HPF with a sensitivity = 71% and specificity = 82%. When dividing the patients according to median alphallb, alphallb-low patients had a median of 0 eosinophils/HPF and alphallb-high patients had a median of 31 eosinophils/HPF ( $p = 0.0006$ , Mann-Whitney test).

**Conclusions:** Platelet association with blood eosinophils, reported by alphallb integrin, after EoE treatment predicted residual PEC with high sensitivity and specificity. The results indicate that a pathway of platelet activation may be predictive of disease activity. Alphallb integrin (CD41) on circulating eosinophils is a potential non-invasive biomarker for disease activity in EoE.

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# Poster Abstract Session 1: Wednesday, 10 July 2019

1

## Eosinophil Infiltration Does Not Exacerbate Muscle Pathology in the MDX Mouse Model of Duchenne Muscular Dystrophy

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**Background:** Duchenne Muscular Dystrophy (DMD) is an inherited disorder of progressive muscle degeneration accompanied by chronic inflammation. Eosinophils are prominent among the leukocytes recruited to the dystrophic muscle tissue, although their contribution to disease pathogenesis remains incompletely understood. In this study, we evaluated the recruitment and impact of eosinophil infiltration on dystrophic muscle damage in the mouse model of disease.

**Methods:** The mdx mice harbor a null mutation in the dystrophin gene and phenocopy the disease seen in DMD patients. We generated mdx-PHIL mice which harbor the null mutation in the dystrophin gene and are congenitally devoid of eosinophils. We also generated mdx-IL5Tg mice, which harbor the dystrophin mutation and have increased levels of eosinophils in circulation and in the muscle tissue. We collected blood, bone marrow, and hind limb muscles from male mice at 4-5 weeks of age, a timepoint that corresponds with the onset of acute muscle injury. We quantified the level of eosinophils in circulation as well as in the bone marrow and in the muscle (CD45<sup>+</sup>CD11c<sup>+</sup>Gr1<sup>+</sup>SigF<sup>+</sup>MHC-II<sup>lo</sup> cells). To evaluate muscle pathology, we scored hematoxylin and eosin-stained muscle sections based on six criteria: myofiber degeneration, myofiber regeneration, myofiber mineralization, leukocyte infiltration, fibrosis and edema. As an additional measure of muscle damage, we measured and compared the peripheral levels of creatine kinase, a marker for muscle damage.

**Results:** We determined that the degree of eosinophil infiltration in dystrophic muscle tissue did not correlate with the degree of muscle damage. First, absence of eosinophils in the muscle tissues of mdx-PHIL mice resulted in a small reduction in the degree of muscle pathology compared to what was observed in mdx mice, and had no impact on the elevated level of serum creatine kinase. By contrast, inclusion of the IL-5 transgene in mdx mice (mdx-IL5Tg) resulted in a 16-fold increase in the number of eosinophils detected in the muscle tissue. Addition of eosinophils likewise resulted in minimal impact on muscle pathology and contributed to a slight but significant *decrease* in the level of serum creatine kinase ( $p < 0.05$ ). We determined that eosinophil infiltration into the muscle tissue of mdx mice was not associated with a systemic eosinophilia or any elevations in Th2 cytokines (IL-4, IL-5, IL-13). However, we did detect significant elevations in eosinophil chemoattractants Eotaxin-1 and RANTES in the muscle tissues of mdx mice at 4 – 5 weeks of age when compared with age-matched wild-type controls.

**Conclusions:** Our findings indicate that eosinophil infiltration in dystrophic muscle tissue is most likely mediated by local production of Eotaxin-1 and RANTES, rather than Th2 cytokines. Furthermore, our data indicate that eosinophil infiltration in the dystrophic muscle tissue does not significantly contribute to acute muscle damage. These findings suggest that eosinophils recruited to the muscle tissue of mdx mice are less likely to be cytotoxic, but may have critical, immunomodulatory properties in this disease process.

**Funding:** This work was funded by the NIAID Division of Intramural Research (#AI000941 to HFR).

3

## In Vitro and In Vivo Inhibition of Eosinophil Activation by Lysophosphatidylcholine and Synthetic Alkyl-Lysophosphatidylcholine

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**Background:** Eosinophils are pleiotropic multifunctional granulocytes involved in initiation and propagation of inflammation. During allergic inflammation eosinophils are vastly recruited to inflamed sites, where they encounter a bioactive lipid lysophosphatidylcholine (LPC). LPC is, however, metabolically not stable and synthetic analogues of the molecule were synthesized to study its function in an *in vivo* setting. One of such is miltefosine- an orally bioavailable alkyl-lysophospholipid. Currently miltefosine has an orphan drug status for the treatment of a tropical disease leishmaniasis and amebic encephalitis. Several groups proved miltefosine acts anti-inflammatory via modulation of

# Poster Abstract Session 1: Wednesday, 10 July 2019

lipid raft mediated signaling in mast cells and T cells. As of yet effects of miltefosine and endogenous LPC on eosinophils in allergic inflammation are not known.

**Methods:** Isolated eosinophils from human blood donors were preincubated with miltefosine or LPC in different concentrations, activated and tested for eosinophil degranulation, migration and CD11b upregulation by flow cytometry. Intracellular signaling including kinase phosphorylation and  $Ca^{2+}$  flux were measured by flow cytometry. For the *in vivo* model of allergic lung inflammation 6-8 weeks old Balb/c mice were sensitized i.p. with ovalbumin in the presence of aluminum hydroxide as adjuvant on days 1 and 7. Furthermore, on days 14 and 16 the mice were challenged with ovalbumin in aerosol to induce allergic inflammation. Between the days 7-17 the mice received either miltefosine or saline with oral gavage. On the last day of the protocol lung function testing was performed and BAL (bronchoalveolar) fluid was taken, stained and analyzed for immune cell composition by flow cytometry. Finally *in vivo* migration of eosinophils in the airways was tested in IL-5 Tg mice.

**Results:** Short pretreatment of isolated eosinophils from human donors with saturated LPCs and miltefosine dose-dependently inhibited their shape change and Cd11b upregulation. Moreover, LPCs and miltefosine decreased eosinophil degranulation and suppressed their migration. Intracellular signaling assays show, that LPC and miltefosine decrease  $Ca^{2+}$  flux and Akt kinase phosphorylation. In an *in vivo* model of ovalbumin induced allergic lung inflammation per os treatment with miltefosine significantly reduced infiltrating immune cells in particular eosinophils, T cells and B cells as well as improved lung function of mice as measured by the methacholine provocation test. Both miltefosine and LPC treatment significantly reduced the number of infiltrating eosinophils in the airways of IL-5 Tg mice.

**Conclusion:** In summary, LPC and Miltefosine suppress eosinophil activation and effector functions following different stimuli, due to impaired intracellular signaling. The *in vitro* effects of miltefosine on eosinophils are translated in an *in vivo* model of allergic lung inflammation, where miltefosine reduces the infiltration of eosinophils in the lung. Since eosinophils are key cells involved in allergic inflammation and propagators of Th2 immune responses, discovery of novel modes of dampening their over-activation, for instance lipid raft modulation, is of critical importance for drug development and future therapeutic intervention.

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## 5

### Characterizing SIGLECF<sup>+</sup>GR1<sup>-</sup> and SIGLECF<sup>+</sup>GR1<sup>+</sup> Mouse Bone Marrow-Derived Eosinophils (bmEos) Cultured *Ex Vivo*

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**Background:** Tissue eosinophils have traditionally been perceived as end-stage effector cells that act as mediators of respiratory allergy and asthma. More recent findings support the concept of heterogeneity of tissue eosinophils with respect to their functions, gene expression and granule contents. While most mouse eosinophils do not express GPI-linked Ly6 antigens detected by the anti-Gr1 antibody, Percopo and colleagues (JLB 2017) identified a small but distinct subpopulation of SiglecF<sup>+</sup>Gr1<sup>hi</sup> eosinophils in the lungs of allergen-challenged mice with a unique cytokine profile. In this study, we utilize eosinophils generated *ex vivo* from unselected bone marrow progenitors to characterize distinct populations of SiglecF<sup>+</sup>Gr1<sup>-</sup> and SiglecF<sup>+</sup>Gr1<sup>+</sup> bone marrow-derived eosinophils.

**Methods:** Unselected bone marrow progenitor cells were isolated from wild type BALB/c mice and used to generate bmEos in *ex vivo* culture as described by Dyer and colleagues (J Immunol 2008). On day 11 of culture, bmEos (99% of total cells as determined by morphological analyses) were separated into Gr1<sup>-</sup> and Gr1<sup>+</sup> populations via fluorescent activated cell sorting (FACS). Cytospin preparations were prepared as well as cell lysates from sorted Gr1<sup>-</sup> and Gr1<sup>+</sup> bmEos, which were analyzed via proteome profiling (Cytokine Panel A, R&D Systems) and DuoSet ELISAs (R&D Systems). RNA was extracted from sorted Gr1<sup>-</sup> and Gr1<sup>+</sup> bmEos and qPCR was performed on synthesized cDNA to evaluate differential gene expression. Finally, Gr1<sup>-</sup> and Gr1<sup>+</sup> bmEos were returned to IL-5 enriched (10 ng/mL) culture medium and evaluated for Gr1, as well as Ly6C and Ly6G expression at subsequent time points.

**Results:** IL-16 and TREM-1 concentrations in cell lysates were upregulated in Gr1<sup>+</sup> bmEos compared to Gr1<sup>-</sup> bmEos (1.2- and 4.7-fold, respectively), while ICAM-1 was downregulated (0.8-fold). GATA-1 was upregulated in Gr1<sup>+</sup> bmEos compared to Gr1<sup>-</sup> bmEos (RQ: 2.8), while mMBP and mEPO were downregulated in Gr1<sup>+</sup> bmEos compared to Gr1<sup>-</sup> bmEos (RQ: 0.27 and 0.07, respectively). Expression of Gr1 increased over time after sorted Gr1<sup>-</sup> bmEos were returned to the IL-5 enriched culture. On day 0 post sort, prior to return to culture, >95% of sorted Gr1<sup>-</sup> bmEos were Gr1<sup>-</sup> and <1% were Gr1<sup>+</sup>. However, on day 5 post sort and return to culture, only 8% of the Gr1<sup>-</sup> bmEos that were originally Gr1<sup>-</sup> remained so; 87% of these cells were now Gr1<sup>+</sup>. Furthermore, most Gr1-expressing bmEos were Ly6G<sup>+</sup> (~80%); many fewer were Ly6C<sup>+</sup> (~8%) or double-positive (~6%). Interestingly, these profiles did not change over time.

**Conclusions:** Our findings suggest that the expression of GPI-linked Ly6 antigens correlates with eosinophil maturation over time in the IL-5 enriched *ex vivo* culture system. The differential expression of various genes, cytokines and granule contents between sorted Gr1<sup>-</sup> and Gr1<sup>+</sup> bmEos supports the case for eosinophil heterogeneity as observed *in vivo*.

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# Poster Abstract Session 1: Wednesday, 10 July 2019

9

## Nasal Polyps as a Presenting Sign of Systemic Eosinophilic Diseases

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**Background:** Chronic sinusitis with nasal polyposis can be a presenting sign of a systemic eosinophilic disease as systemic vasculitis or hypereosinophilic syndrome. In other cases it might masquerade additional eosinophilic comorbidities including eosinophilic asthma, or allergic fungal sinusitis.

**Aim:** To evaluate patients that are candidates for surgical intervention by functional endoscopic sinus surgery (FESS) due to severe sinusitis and nasal polyposis, for undiagnosed systemic eosinophilic diseases. Consequently, to improve treatment and outcome of these comorbidities when found.

**Methods:** patients with severe chronic sinusitis with nasal polyposis were referred to the allergy clinic for farther evaluation. All patients underwent a thorough clinical and laboratory assessment as well as quality of life assessment (measured by the SNOT 21 questioner). A special emphasis was given to lung function tests, skin tests to molds and fungi, biopsies for vasculitis and genetic workup as indicated in hypereosinophilic syndrome.

**Results:** Twenty males and 33 females were included in our study. All suffered from severe chronic sinusitis with nasal polyposis and were candidate for surgical intervention by functional endoscopic sinus surgery (FESS). The average age of our patients was  $45.1 \pm 15$  (range 16-82). Thirty eight (71.6%) patients were diagnosed with eosinophilic asthma according to their history and lung function tests. Most of them (32/38, 84.2%) were undertreated or with uncontrolled asthma. Biopsies and skin tests confirmed the diagnosis of allergic fungal sinusitis in four (7.5%) patients. According to the current criteria - Eosinophilic Granulomatosis with polyangiitis (EGPA) was found in 2 (3.7%) patients and hypereosinophilic syndrome in two patients (3.7%, one with the myeloid variant and the FIP1L1 mutation and one with a lymphocytic variant). The average eosinophil count in the blood was  $1340 \pm 1600$ , with evidence of eosinophilic inflammation in 36 patients (68%) and severe eosinophilic inflammation in 15 patients (28.3%).

Specific biological treatment (Anti IGE or Anti IL5) was indicated and recommended in 26 patients (49%).

After a mean follow-up of 12 months only one patient (1.9%) underwent surgery.

Quality of life has improved significantly at the end of the follow up period ( $50.8 \pm 26.87$  vs  $25.6 \pm 9.2$ ,  $p=0.05$ ).

**Conclusion:** In patients with chronic sinusitis with nasal polyposis, a thorough evaluation for eosinophilic comorbidities is indicated. This improves quality of life, disease control specific measures, precision of medical treatment as well as avoidance of non-essential surgery.

11

## Dexramipexole Responsiveness is Increased in Eosinophilic Patients

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**Background:** Dexramipexole (DexP) eosinophil lowering is greater in Chronic Rhinosinusitis with Nasal Polyps than in Amyotrophic Lateral Sclerosis (ALS). We sought to determine if DexP eosinophil lowering is affected by patients' eosinophilia.

**Methods:** Patient level data from the 223AS302 randomized, double-blind, placebo-controlled study of DexP in ALS were retrospectively analyzed to assess eosinophil lowering as a function of baseline absolute eosinophil count (AEC). Eosinophil reduction ratio (RR) was calculated as the mean of AEC values in Months 4-6 divided by the baseline visit AEC. The eosinophil lowering response rate was quantified as the percentage of subjects achieving an eosinophil RR less than or equal to 0.25 or 0.10.

**Results:** Eosinophil lowering response rates were minimal in the stratum with the lowest baseline AEC. There was a step-wise increase in eosinophil lowering response in strata with greater baseline AEC, plateauing in strata with  $AEC \geq 0.25 \times 10^9/L$ .

|                                  | Entire study population | Study population stratified by baseline AEC ( $\times 10^9/L$ ) |           |           |           |           |           |           |             |
|----------------------------------|-------------------------|---|-----------|-----------|-----------|-----------|-----------|-----------|-------------|
|                                  |                         | <0.05   | 0.05-0.90 | 0.10-0.14 | 0.15-0.19 | 0.20-0.24 | 0.25-0.29 | 0.30-0.34 | $\geq 0.35$ |
| # subjects                       | 445                     | 35  | 144       | 113       | 73        | 39        | 19        | 8         | 14          |
| Subjects with RR $\leq 0.25$ (%) | 49.2                    | 8.6   | 32.6      | 55.8      | 71.2      | 61.5      | 73.7      | 75.0      | 71.4        |



# Poster Abstract Session 1: Wednesday, 10 July 2019

|                                  |      |      |      |      |      |      |      |      |      |
|----------------------------------|------|------|------|------|------|------|------|------|------|
| Subjects with RR $\leq$ 0.10 (%) | 18.0 | 2.86 | 5.56 | 22.1 | 19.2 | 25.6 | 47.4 | 75.0 | 50.0 |
|----------------------------------|------|------|------|------|------|------|------|------|------|

**Conclusions:** These data demonstrate that DexP-induced eosinophil lowering increases in a stepwise manner in subjects with higher baseline eosinophil counts. Conversely, eosinophil lowering was modest in subjects without eosinophilia (AEC  $\leq$ 0.10x10<sup>9</sup>/L). Subjects with a baseline AEC  $\geq$ 0.25x10<sup>9</sup>/L had an eosinophil lowering response rate of 70-75%. These findings suggest that similar high rates of dexpropimexole responsiveness may be observed in eosinophilic asthma. These findings are consistent with DexP acting on a specific pathway(s) driving eosinophilia, but not affecting homeostatic eosinophil production.

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13

## Single Site, Five-Year Experience with Human Eosinophil Isolation by Density Gradient Centrifugation and Immunomagnetic Negative Separation

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**Background:** Several methods have been developed for the purification of human eosinophils, but little is known about their reproducibility, yield, reliability and safety. We have retrospectively reviewed our experience with 350 consecutive eosinophil isolation preparations and describe our observations.

**Methods:** EDTA-anticoagulated blood (25–180 mL) was diluted 1:3 with PBS, layered onto 10 mL cushions of Percoll adjusted to a specific gravity of 1.090 and centrifuged at 335 x g for 20 minutes at room temperature without braking. Mononuclear cells, platelets and basophils in the upper layer were removed. Granulocytes and RBCs were collected from the pellet, and RBCs were lysed with ice cold water for 30 seconds. Finally, anti-human CD16 magnetic microbeads were added to label neutrophils and negatively select eosinophils over a magnetized mesh column. For most blood donations, a complete blood count (CBC) with differential was drawn simultaneously and analyzed by our hospital laboratory.

**Results:** Between January 2014 and December 2018, we conducted 350 eosinophil purifications from 83 donors (24 males, 59 females, age 21–60 with a mean age of 30; 46 were allergic and 37 were non-allergic based on medical and medication history). Absolute eosinophil count (AEC), calculated from the CBC results when drawn (n=289), ranged from 32 to 1352 eosinophils/ $\mu$ L ( $\pm$  SD: 179  $\pm$  136/ $\mu$ L). Eosinophil yields after purification per 20 mL of blood drawn ranged from 0.4 to 13.3 million cells ( $\pm$  SD: 3.1  $\pm$  1.9 million eosinophils) with >95% purity. Comparing AEC to actual yield, recovery was 87%  $\pm$  29% ( $\pm$  SD) with a strong linear correlation between AEC and yield (Pearson  $r$  =0.897). To explore the reproducibility of yield, a subsequent analysis was limited to those donors that were drawn  $\geq$ 3 times (N=38). With this approach, the average coefficient of variation for yield did not differ between allergic (N=25) and non-allergic (N=13) donors (40% versus 38%, respectively), suggesting that there was no detectable impact of seasonal allergy on eosinophil yield. Initial viability of isolated eosinophils was consistently >95% and declined to >90% after 24 hr. of culture with 30 ng/mL rhIL-5 versus >80% without IL-5, and did not differ between allergic and non-allergic donors. Overall safety was excellent, although we did note the following from the CBC results: mild lymphopenia (n=1), neutropenia (n=4) or thrombocytopenia (n=2); anemia (n=4); slight ovalocytes (n=1), and large platelets (n=1).

**Summary and Conclusions:** The described method for human eosinophil isolation from whole blood using density gradient centrifugation, RBC hypotonic lysis and immunomagnetic removal of neutrophils is reliable, reproducible and safe for obtaining an average of 87% yield with high purity and viability.

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15

## Genetic Variation in Surfactant Protein-A2 and the Effect on Eosinophil Resolution in Allergic Airways

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**Background:** Several recent reports demonstrate the importance of surfactant protein-A in asthma. There are two human SP-A genes, *SP-A1* and *SP-A2*, which combine to form higher order oligomeric structures. While many genetic variants of SP-A exist, a specific genetic variant of SP-A2 (rs1965708), corresponding to a Q(Gln) to K(Lys) amino acid substitution at position 223 of the lectin domain, is associated with lower lung function in asthmatic patients. Studies from our lab show differential effects of SP-A2 based on the Q223K poly-

# Poster Abstract Session 1: Wednesday, 10 July 2019

morphism on eosinophil activities. Since eosinophilia is a well-documented phenotype in a large subgroup of asthmatics, we wanted to determine the effect of genetic variation at position 223 of SP-A2 on eosinophil clearance from allergic airways.

**Methods:** Purified eosinophils from the peripheral blood of IL-5 transgenic mice were isolated to assess the effects of SP-A2 genetic variation on eosinophil chemotaxis and cell death *in vitro*. WT, SP-A<sup>-/-</sup> and humanized (SP-A2 223Q/Q, SP-A2 223K/K) mice were challenged using an allergic OVA model and eosinophilia was examined 24 hours and 5 days after terminal challenge.

**Results:** *In vitro*, human SP-A carrying either the 223Q/Q or the 223Q/K allele was chemoattractant for eosinophils. In contrast, upon direct incubation of eosinophils only SP-A 223Q/Q significantly increased cell death compared to vehicle control. *In vivo*, SP-A<sup>-/-</sup> and humanized SP-A2 223K/K mice had persistent eosinophilia in the bronchoalveolar lavage (BAL) from 24 hours to 5 days post-terminal challenge (d.p.c.), whereas the humanized 223Q/Q mice had significantly decreased eosinophil numbers by day 5. Comparing tissue eosinophilia between the respective genotypes 24 hours and 5 days post challenge, humanized SP-A2 223Q/Q and SP-A2 223K/K mice had significantly decreased eosinophilia in tissue as compared to SP-A<sup>-/-</sup> mice.

**Conclusions:** Our results suggest that SP-A, regardless of the Q223K polymorphism, promotes eosinophil chemotaxis from lung tissue to the lung lumen, the absence of which results in persistent tissue eosinophilia. In the lung lumen, SP-A, depending on the Q223K polymorphism, promotes eosinophil death, the absence of which or presence of an altered isoform results in persistent airway eosinophilia.

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17

## Eosinophil Cytolysis on IGG Requires Microtubule Array Formation, and P-38 Phosphorylation Downstream of Reactive Oxygen Species Production

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**Background:** The presence of eosinophils in the airway is associated with asthma severity. We have recently shown that treatment with a therapeutic anti-IL-5 monoclonal antibody followed by a segmental allergen challenge in human subjects with mild asthma, led to significant reduction of intact eosinophils in the airways. Yet, deposition of eosinophil granule proteins and intact eosinophil granules were clearly present in the lung tissue despite anti-IL-5 treatment. These observations indicate that eosinophils have lysed and thus released their toxic products, including free molecules and intact granules in the airway. Previously, eosinophil membrane disruption and the release of granules in tissues has been observed in human allergic diseases. Notably, intact granules further release toxic proteins, even when eosinophils are absent. Thus, eosinophil lysis, in contrast to eosinophil clearance by apoptosis or para-epithelial migration, may be an important mechanism for damaging tissue in patients with eosinophilic diseases. Therefore, it is important to understand the mechanisms leading to cytolysis to ultimately block the discharge of eosinophilic compounds, intact granules, and consequent delayed release of damaging eosinophilic toxic agents in tissues.

**Methods:** We have developed an alphaMbeta2 integrin-dependent *in vitro* model leading to strong eosinophil degranulation and apparent cytolysis on aggregated immunoglobulin G (IgG). In this model, human circulating eosinophils were primed with IL3 and were subsequently seeded on coated IgG. This same experimental design was used in the current study to measure adhesion, degranulation (EDN release) and cytolysis. Cytoskeletal events and signaling pathways involved in cytolysis were screened using inhibitors added after IL3 priming. Next, we measured the level of activation of the identified events and pathways specifically involved in cytolysis, and we analyzed the potential cross talk between these pathways.

**Results:** We found that PI3K controls eosinophil adhesion and degranulation on IgG. Actin polymerization is not required for adhesion but significantly participated in degranulation. Eosinophil cytolysis was dependent on production of reactive oxygen species (ROS) and downstream, p-38 phosphorylation. In addition, formation of microtubule arrays was necessary for cytolysis. The blockade of cytolysis by ROS and p-38 inhibitors led to large microtubule arrays, suggesting that microtubule dynamic changes may be involved in preventing eosinophil lysis. ROS, p-38 phosphorylation, or microtubule formation did not significantly affect adhesion and degranulation.

**Conclusions:** In this study, we showed that cytolysing eosinophils displayed microtubule arrays, production of ROS, and p-38 phosphorylation when seeded on IgG. We anticipate that inhibition of any of these pathways would block eosinophil cytolysis and subsequent eosinophil-driven damage in tissues.

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# Poster Abstract Session 1: Wednesday, 10 July 2019

19

## IL-9 and IL-13 Storage and Release from Eosinophils

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**Background:** Asthma is a major chronic disease affecting a significant proportion of the population, and ~50% of asthmatics exhibit elevated tissue and blood eosinophils. Eosinophils synthesize and release T helper 2 (Th2) cytokines, including interleukin-9 (IL-9) and IL-13, that skew the immune response to an allergic phenotype. Intracellular localization of IL-9 is not yet confirmed, while IL-13 has been shown to localize to crystalloid granules in eosinophils. IL-9 has a role in promoting allergic Th2 responses, while IL-13 is a pro-fibrotic cytokine important for tissue remodeling. Exactly where IL-9 and IL-13 are stored in eosinophils and their trafficking mechanisms remain unknown. Here, we hypothesize that IL-9 and IL-13 are found as pre-formed cytokines stored in eosinophil crystalloid granules and other intracellular organelles, and are secreted using distinct trafficking pathways.

**Methods:** Human peripheral blood eosinophils were purified from venous blood of mildly eosinophilic volunteers by MACSxpress. Eosinophils were adhered to glass coverslips and stimulated with 5  $\mu$ M platelet activating factor (PAF), a potent secretagogue. Cells were immunolabeled with antibodies specific to IL-9 or IL-13 and colocalized with markers for secretory organelles (CD63 for crystalloid granules, transferrin receptor [TfnRc] for recycling endosomes). We imaged cells using Deltavision OMX super resolution microscopy and quantified colocalization by Pearson's correlation coefficient using Volocity. We created a protocol for automated colocalization, which generated similar results to manual colocalization, and applied this approach.

**Results:** Colocalization of IL-9 and CD63 significantly increased from  $0.50 \pm 0.01$  to  $0.57 \pm 0.02$  after 5 min of PAF stimulation, but decreased to  $0.45 \pm 0.01$  after 60 min ( $p < 0.01$ ). However, colocalization with IL-13 with CD63 was  $0.37 \pm 0.01$ , with no significant changes in resting vs stimulated cells. Colocalization of IL-9 with TfnRc increased from  $0.47 \pm 0.01$  to  $0.56 \pm 0.01$  after 5 min PAF stimulation, and continued to increase to  $0.60 \pm 0.008$  after 60 min ( $p < 0.0001$ ). In contrast, colocalization of IL-13 with TfnRc increased following 5 min stimulation from  $0.41 \pm 0.02$  to  $0.43 \pm 0.01$  ( $p < 0.001$ ), but then decreased to  $0.39 \pm 0.01$  ( $p < 0.05$ ) after 60 min. The intensity of IL-9 detected in the cell increased from 0 to 5 minutes ( $p < 0.001$ ) and at every time point thereafter ( $p < 0.0001$ ). While IL-13 intensity initially increased at 5 min ( $p < 0.001$ ), it decreased at 15 and 60 min PAF stimulation ( $p < 0.0001$ ).

**Conclusions:** These results indicate that both IL-9 and IL-13 are stored in crystalloid granules, while only IL-9 is released using TfnRc<sup>+</sup> recycling endosomes. Increased IL-9 detection suggests *de novo* synthesis of this cytokine when eosinophils are stimulated, while IL-13 levels decreased, suggesting loss of preformed IL-13. Understanding these differentiated mechanisms involved in IL-9 and IL-13 release will contribute to our understanding of cytokine secretion, and coordinated efforts will link mechanistic understanding of cytokine release with clinical practice in treating patients with eosinophilic asthma.

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21

## The Role of Eosinophils in Obesity-Related Asthma

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**Background:** Eosinophils have been identified as important immune cells with both regulatory and inflammatory characteristics. They are known to induce airway hyperresponsiveness, an abnormal bronchoconstriction response to inhaled stimuli, as well as play a role in metabolic homeostasis. Obesity-related asthma is one state of metabolic dysfunction in which the heterogeneous activity of eosinophils in the lung and adipose tissue is unclear. Here we tested the effect of increased circulating eosinophils on the lungs and adipose tissue using a diet-induced obese mouse model.

**Methods:** Wild type C57BL/6J mice (WT), transgenic NJ1638 mice with systemic eosinophilia driven by high IL5 (+Eos), and NJ1638/Phil mice with eosinophil-deficiency, but high IL5 (-Eos) were fed a normal or high fat diet (HFD) for 19 weeks. Food intake, body weight, body fat composition, and fasting glucose were measured. Airway responsiveness to inhaled serotonin, which induces vagally-mediated reflex bronchoconstriction, was tested in fasted, ventilated, and anesthetized mice. To isolate eosinophils, eosinophil-specific green fluorescent protein-expressing mice (EoCRE<sup>+/+</sup>GFP<sup>+/+</sup>) were crossed with WT and +Eos mice, and the offspring were fed a normal or high fat diet for 19 weeks. Blood, adipose, and lung tissue eosinophils were sorted by flow cytometry.

**Results:** Body fat and airway responses to aerosolized serotonin were similar between WT, +Eos, and -Eos mice fed a normal diet. When fed HFD, WT and +Eos mice consumed similar amounts of food. Despite this, WT mice gained more weight, had higher body fat composition, and had

## Poster Abstract Session 1: Wednesday, 10 July 2019

higher fasting blood glucose than +Eos mice. WT mice on a HFD developed significant airway hyperresponsiveness to serotonin, while +Eos mice on a HFD did not. Using flow cytometry to count eosinophils in blood, adipose and lung tissue, +Eos mice on normal diet had more eosinophils per gram lung tissue and in blood compared to WT mice but the same number of eosinophils per gram adipose tissue. On HFD, +Eos mice had increases in eosinophils in all tissues compared to WT mice, although HFD +Eos had overall fewer eosinophils per gram than +Eos on normal diet.

**Conclusions:** An increase in circulating eosinophils was associated with lower body fat composition and reduced airway responsiveness in response to HFD. Differences in eosinophil phenotypes, recruitment, or activation may regulate body fat and may also protect against airway hyperresponsiveness in obesity.

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23

### Kinetic Studies of Galectin-10 Release from Eosinophils to Proliferating T Cells

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Eosinophils suppress T cells in part by using galectin-10, but it is unknown how galectin-10 is released from eosinophils to T cells. Our primary goal was to determine how galectin-10 is released from eosinophils exposed to proliferating T cells as well as the kinetics behind the galectin-10 release. Human eosinophils were cocultured with CD3/CD28-activated T cells on microscope slides for 30, 60, 120 minutes, and the cells were then stained for galectin-10, actin and DNA and further analyzed with confocal microscopy. We discovered that eosinophils cocultured with proliferating T cells have a distinct kinetic pattern when releasing galectin-10. At 30 minutes, galectin-10-containing synapses formed between eosinophils and T cells was seen. At 60 minutes, the plasma membrane of eosinophils began to disintegrate and the bi-lobulated nucleus was lost. Simultaneously, cap-like accumulations of galectin-10 budded on the eosinophil cell surface. Interestingly, the galectin-10 caps did not form randomly, but were always placed in front of the nuclear lobes. Lastly at 120 minutes, massive release of eosinophil extracellular traps composed of nuclear DNA and galectin-10 were freed. To conclude, eosinophils appear to release galectin-10 in a strict kinetic manner when suppressing proliferating T cells.

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25

### Eosinophil Responses to IL-4, IL-33 and Bacteria are Differentially Regulated By Apoptotic Cells

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**Background:** Eosinophils are present in mucosal surfaces such as the gastrointestinal and respiratory tracts. In these tissues, they are associated with multiple inflammatory diseases that are characterized by the increased presence of apoptotic cells (ACs). Clearance of ACs triggers a potent anti-inflammatory response that serves as a fundamental basis for resolution of inflammation. Although eosinophils are associated with tissue repair and are likely present in the proximity of multiple ACs, the effects of ACs on eosinophils are largely unexplored.

**Methods:** Expression of cell surface receptors that can recognize the major AC marker, phosphatidylserine was determined by qPCR and flow cytometry. Eosinophils were obtained from the following sources: bone marrow-derived eosinophils were obtained from wild type, *Cd300b*<sup>-/-</sup> or *Cd300f*<sup>-/-</sup> mice. Peritoneal eosinophils were obtained from *I15Tg* mice. Thereafter, the eosinophils were stimulated with ACs in the presence of heat inactivated *E. coli*, IL-4 or IL-33. Cytokines secretion in the cell supernatants was determined by ELISA.

**Results:** ACs are recognized by multiple receptors including those belonging to the CD300 family members, T-cell immunoglobulin and mucin domain-containing (TIM)-4, Tyro3, Axl, and MerTk. First, we aimed to define whether eosinophils express receptors that enable them to respond to ACs. Peritoneal eosinophils expressed multiple CD300 family receptors and TIM4 but not Tyro3, Axl, and MerTk. Activation of eosinophils with *E. coli* resulted in markedly elevated secretion of IL-6 and TNF- $\alpha$ . Activation of eosinophils with *E. coli* in the presence of ACs resulted in near complete suppression of proinflammatory cytokine secretion and markedly suppressed the phosphorylation of JNK. Similarly, ACs were capable of abrogating IL-33-induced proinflammatory cytokine secretion from eosinophils. Strikingly, and in contrast to the inhibitory effect of ACs on IL-33-induced eosinophil activation, IL-4-induced eosinophil activation was markedly increased in the presence of apoptotic cells. Importantly, apoptotic bodies and supernatants that were obtained from apoptotic cells (without apoptotic bodies) differentially regulated eosinophil activation in response to *E. coli*, IL-33 and IL-4 as well. In support of this, CD300f and CD300b had no role in the effects of ACs on *E. coli*, IL-33- and IL-4-induced eosinophil activation. Furthermore, modulation of eosinophil activation was likely independent of AC engulfment since eosinophils displayed limited engulfment abilities.

**Conclusion:** Our data highlights ACs as potent regulators context-dependent regulators of eosinophil activities.



# Poster Abstract Session 1: Wednesday, 10 July 2019

27

## Harnessing *Hymenolepis Diminuta* Extract to Active Anti-Tumorigenic Activities of Eosinophils in Colorectal Cancer

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**Background:** Colorectal cancer (CRC) is the third most commonly diagnosed cancer and one of the major leading causes of cancer-associated death worldwide. Despite major advances in immunotherapy, it has been effective only in subsets of CRC patients, highlighting the urgent need to new treatment modalities. We have recently demonstrated potent anti-tumorigenic activities for eosinophils in CRC. Therefore, as a future therapeutic strategy, we aimed to identify mechanisms that could utilize their anti-tumorigenic activities.

**Methods:** *Hymenolepis diminuta* extracts (HdAg) were obtained by homogenization in sterile PBS. Subcutaneous CRC tumors were induced by injection of MC38 CRC cells ( $5 \times 10^5$  cells/mouse) to wild type mice and  $\Delta$ dblGATA mice. HdAg was injected intraperitoneally or intratumorally and accumulation of eosinophils, immune phenotyping and tumor burden assessed. Eosinophils were purified from *I57g* mice and stimulated in vitro with HdAg. Thereafter, eosinophil migration, survival and mediator release was determined.

**Results:** Subcutaneous injection of MC38 cells, resulted in markedly increased eosinophilic infiltration into the tumors. In this model, eosinophils displayed anti-tumorigenic activities since injection of MC38 cells into  $\Delta$ dblGATA mice resulted in significantly increased tumor burden in comparison with wild type mice. In order to determine whether HdAg could be potentially used to increase accumulation of eosinophils into the tumor site, we first examined whether intraperitoneal injection of HdAg increased local and/or systemic eosinophilia. Certainly, intraperitoneal injection of HdAg into WT mice resulted in significantly increased dose-dependent accumulation of eosinophils in the peripheral blood and peritoneal cavity, which was evident as early as 24 h after injection and lasted for 72 hrs. Furthermore, intratumoral injection of HdAg into subcutaneous seeded of MC38 cells resulted in significantly decreased tumor burden, which was associated with substantially increased infiltration of eosinophils and CD4<sup>+</sup> T cells. Notably, HdAg had no direct effect on eosinophil survival, migration and mediator release.

**Conclusion:** We demonstrate that eosinophils display anti-tumorigenic effects in CRC. Furthermore we show that HdAg, could induce a Th2 immune response that is characterized by eosinophilia and CD4<sup>+</sup>, and is associated with tumor reduction. These data open potential new avenues for treatment of solid tumors such as CRC by harnessing Th2 immunity and eosinophils.

29

## Granulocyte Kinetics in the Blood and Sputum of Patients with Eosinophilic and Neutrophilic Inflammation

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**Background:** Little is known about the kinetics of inflammatory cells in blood and sputum of patients with different inflammatory lung diseases ranging from eosinophil dominated disease in eosinophilic asthma to neutrophil dominated disease such as found in cystic fibrosis. The life spans of inflammatory cells in the sputum in humans is unknown.

**Methods:** The kinetics of granulocytes in circulation and sputum were studied by labelling 11 healthy volunteers, 6 cystic fibrosis (CF) patients and 9 eosinophilic asthma (EA) patients with 6,6-<sup>2</sup>H<sub>2</sub>-glucose for 6 hours. Neutrophils, eosinophils and free DNA (only in CF sputum) were isolated from both blood and sputum by FACS sorting at different time points, and DNA 2H-enrichment was determined by GC-MS. Hereafter, mathematical modeling was applied to determine half lives in the blood and transit times in the sputum.

**Results:** The neutrophil compartment in CF patients exhibited a ~1 day shorter maturation time in bone marrow compared to healthy controls, while the neutrophil compartment of EA patients was unaffected. In contrast, the eosinophil compartment in EA patients was characterized by a ~1 day longer maturation time in BM compared to healthy controls, while the dynamics of eosinophils of CF patients were marginally affected. In addition, eosinophil turnover in the circulation was significantly faster in EA patients than in healthy controls. While the kinetics of neutrophils in blood and sputum were highly comparable, eosinophils seemed to follow different labelling patterns in blood and sputum. These findings suggest that neutrophils survive only briefly in sputum, while eosinophils in sputum can survive much longer. The <sup>2</sup>H-kinetics of free DNA in the sputum of CF patients was similar to that of neutrophils in blood and sputum, yet with a delay of around 1 day.

**Conclusions:** These findings provide critical insight into the behavior of both neutrophils and eosinophils in blood and lungs during inflammatory conditions. Our data support longevity of eosinophils in the sputum of EA and CF patients, but contrast the widely held view that neutrophils survive for relatively long periods of time in the sputum of lung patients with neutrophil inflammation.

**Grant Support:** The study was supported by research grants from the Dutch Lung Foundation and the Dutch CF Foundation.

## IGG4-Related Disease and Hypereosinophilic Syndrome: Overlapping Phenotypes?

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**Background:** IgG4-related disease (IgG4-RD) is associated with peripheral eosinophilia in 10-30% of cases, possibly due to a Th2-driven immune response. A preliminary study suggested that disease features of IgG4-RD and lymphocyte variant-hypereosinophilic syndrome (HES) could overlap. Yet, no reference to IgG4-RD was made in the latest classification criteria for eosinophilic disorders and related syndromes established by the International Cooperative Working Group on Eosinophil Disorders (ICOG-Eo).

**Methods:** We investigated both the French IgG4-RD Study Group and the National Referral Center for Hypereosinophilic Syndromes (CEREO) databases. Patients fulfilling (i) Comprehensive Diagnostic Criteria (CDC) for IgG4-RD (and/or organ-specific diagnostic criteria for IgG4-RD); and presenting with (ii) eosinophilia  $\geq 1$  G/L were included. Data regarding baseline clinical and paraclinical findings as well as treatment responses were retrieved using a standardized form.

**Results:** Thirty-two patients (27 male; median [range] age at diagnosis: 55 [26-79] years), including 21, 7 and 3 patients with definite, probable and possible IgG4-RD respectively were included (a single patient did not have tissue biopsies but had definite autoimmune pancreatitis according to ICDC criteria). The main related IgG4-related involvements included lymph nodes (n=21), salivary glands (n=15), pancreas (n=13), lungs (n=9), bile duct (n=8), kidney (n=8), orbit (n=7), large-vessels (n=6) and retroperitoneal fibrosis (n=5). Twenty-nine (91%) patients had high IgG4 levels (median 7,4 g/L) and all but one (97%) had polyclonal hypergammaglobulinemia (reaching 110g/L in a patient). The median [range] absolute eosinophil count peak was 2 [1-18] G/L and the main eosinophil-related organ damage included lungs (n=10), skin (n=7), ENT (n=7), heart (n=5), gastro-intestinal tract (n=4) and peripheral arterial vasculopathy (n=4). Eighteen (56%) patients had high IgE levels, while vitamin B12 and tryptase were elevated in a single patient (3%). Twenty-seven (84%) patients fulfilled classification criteria for HES, including 2 patients with lymphocyte variant-HES.

Oral corticosteroids were effective against both eosinophil and IgG4-related organ damage in all cases, yet further-line treatments were required for 22 (69%) patients. Mepolizumab was effective against eosinophil-related organ damage in both treated patients but failed to curb IgG4-RD. Rituximab led to a drastic decrease of serum IgG levels, was effective against IgG4-related features in all 9 treated patients, and further enabled complete or partial hematological responses in all patients but one.

**Conclusions:** Some patients concomitantly fulfill criteria for both IgG4-RD and HES. IgG4-RD should be considered as a possible diagnosis in patients presenting with unexplained eosinophilia or eosinophil-related organ damage, especially in the setting of IgG4-RD defining features and/or polyclonal hypergammaglobulinemia. Oral CS are effective on both disease manifestations. When CS-sparing treatments are required, rituximab seems to be a sound therapeutic option. Updated ICOG-Eo classifications should consider IgG4-RD as an associated condition for eosinophilic disorders.

## Allergic Bronchopulmonary Aspergillosis as a Complication of COPD: A New Entity?

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**Background:** To date, allergic broncho-pulmonary aspergillosis (ABPA) is associated with two underlying conditions: asthma and cystic fibrosis. Chronic obstructive pulmonary disease (COPD) disease is not considered as a usual Th2-driven disease and eosinophilia or high levels of IgE are uncommon in this setting. Here, we report on ABPA occurring in patients with long-standing COPD.

**Methods:** Adult patients with diagnoses of both COPD (GOLD criteria) and ABPA (Patterson's criteria) followed in our institution were identified thanks to the medical information system database. Patients' demographic, clinical and paraclinical (including spirometry values and assessment of Bhalla scoring system on chest CT-scans) findings as well as follow-up outcomes were recorded and compared to 16 ABPA-free COPD patients matched on age, FEV1 and follow-up duration.

**Results:** Sixteen patients (13 men) with a median age of 61 (range 55 to 79) years and a median tobacco consumption of 47 PY were identified. The diagnosis of ABPA followed that of COPD after a median of 6 years (with a range from 1 to 10 years). At diagnosis of ABPA, median FEV1 was 36% (1127mL). Median (range) total of both total and *Aspergillus* specific IgE levels as well as peak eosinophil count



## Poster Abstract Session 1: Wednesday, 10 July 2019

were 1450 kU/l [1017-3285], 5.66 kU/l [1.25-35.38], and 0.95 G/L [0.425-0.1725]. Thirteen patients (81%) had positive IgG, 10 (62.5%) had evidence of *Aspergillus sp.* in sputum, while immediate prick tests were positive in 6 out of 7 (86%) patients.

All patients received inhaled and oral steroids, 13 patients received oral fungicides and 7 omalizumab.

During follow-up, compared to ABPA-free COPD patients, ABPA-BPCO patients had significantly higher rates of both annual FEV1 decline (-56 vs -34 mL/year;  $p=0.04$ ) and flare-related hospitalizations (1.69 vs 0.53/year;  $p=0.0007$ ). Bronchiectasis extension ( $p=0.002$ ) and mucoid impaction extension ( $p=0.03$ ) but not emphysema ( $p=0.45$ ) were significantly higher in ABPA-BPCO patients than in ABPA-free COPD patients. Among ABPA-BPCO patients, the use of antifungal drugs prevented the loss of FEV1 (-51 mL/year vs +92 mL/year;  $p=0.02$ ) whereas, albeit not reaching statistical significance, omalizumab tended to lower the annual exacerbations rate (1.35 vs 0.35 /year,  $p=0.12$ ).

**Conclusion:** Pulmonologists should be well aware that eosinophilia occurring in COPD patients can be related to genuine ABPA, which is associated with impaired respiratory function and multiple hospitalizations. Treatment with antifungal drugs and/or omalizumab could improve long-term outcomes and should be discussed promptly once the diagnosis is established.

35

### Intrinsic Sex-Specific Differences in Fibrotic Gene Expression and Pathology Contribute to Male Disease Predisposition in Pediatric Eosinophilic Esophagitis

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**Background:** Eosinophilic esophagitis (EoE) is a chronic allergic disease characterized by esophageal inflammation and fibrosis with a significantly higher disease prevalence in males than females (3:1). The underlying mechanisms behind this sexual dimorphism remain unclear.

**Methods:** Primary fibroblasts were isolated from male and female EoE patients' esophageal biopsies. Normal fibroblasts were isolated from human donor esophagi. EoE patients had active disease. Fibroblasts were treated with recombinant IL-4, IL-13, TGF $\beta$ 1, or vehicle and analyzed for inflammatory and fibrotic gene expression. Esophageal biopsy specimens from active disease patients were scored using a standardized histology score.

**Results:** IL-4 or IL-13 induced significantly more eotaxin-3/CCL26 expression in esophageal fibroblasts from males as compared with females ( $p<0.001$ ). This trend was observed in fibroblasts derived from adults or children, consistent with increased EoE onset in males of all ages. TGF $\beta$ 1 treatment induced significantly higher levels of collagen1,  $\alpha$ -smooth muscle actin, fibronectin, and serpinE1 ( $p<0.03$ ) mRNA in male ( $n=5$ ) EoE esophageal fibroblasts as compared with females ( $n=3$ ). Hematoxylin and eosin stained patient biopsies ( $n=153$ , 125 males and 28 females) revealed that while overall eosinophil count correlated with epithelial remodeling score ( $r=0.3550$ ,  $p<0.0001$ ) within the distal esophagus, this correlation was distinctly stronger in the male patients ( $r=0.4062$ ,  $p<0.0001$ ) than within the female patients ( $r=0.1546$ ,  $p=0.4323$ ). The same pattern was found in the middle (male  $r=0.6080$ ,  $p<0.0001$ ; female  $r=0.4723$ ,  $p=0.013$ ) and proximal esophageal levels (male  $r=0.8504$ ,  $p<0.0001$ ; female  $r=0.5235$ ,  $p=0.006$ ).

**Conclusions:** Male esophageal fibroblasts have a more robust response to Th2 interleukins and TGF $\beta$ 1 as compared with female fibroblasts in adults and children and during the normal and active disease states, suggesting an intrinsic sexual dimorphism in esophageal cells. Epithelial histologic disease severity in males also correlates more strongly with elevated eosinophil counts than in females, suggesting that the male esophagus may have more profound responses to cytokines or antigens as compared to females. These data support a potential mechanism for the sex biased disease expression of EoE.

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37

### Urine Levels of Eosinophil Granule Proteins; a Better Biomarker of Eosinophilic Disease Activity?

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## Poster Abstract Session 1: Wednesday, 10 July 2019

**Background:** Biomarkers of eosinophilic disease activity in the context of novel therapies that deplete eosinophils in the blood are an unmet need. Although measurement of eosinophil granule protein levels in serum or plasma has shown some utility in this regard, the inconvenience of venipuncture and the potential for eosinophil lysis during collection or processing of samples can be problematic. Measurement of granule proteins in urine provides a potential solution to these issues. To begin to explore the utility of this approach, we examined both serum and urine eosinophil granule proteins in the setting of a clinical trial of benralizumab (anti-IL5R $\alpha$ ) for the treatment of hypereosinophilic syndrome (HES).

**Methods:** Serum and urine samples were collected at fixed timepoints from 20 symptomatic subjects with *PDGFRA*-negative HES enrolled on a double-blind, placebo-controlled study of benralizumab (NCT02130882). Serum and urine samples collected at a single time point from healthy volunteers (NCT00090662) were used as experimental controls. All samples were reduced and alkylated prior to quantification of eosinophil granule proteins (major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO)) by multiplex assay. Eosinophil granule protein levels in urine were normalized using specific gravity. All blood samples were centrifuged within 30 minutes of venipuncture.

**Results:** Serum levels of MBP ( $p < 0.0001$ ), EDN ( $P = 0.0230$ ) and EPO ( $p < 0.0001$ ) and urine levels of MBP ( $p = 0.0002$ ) and EDN ( $p < 0.0001$ ) were significantly decreased in HES patients after 3 doses of benralizumab. Whereas baseline serum levels of MBP ( $p = 0.0320$ ,  $r = 0.493$ ), EDN ( $p = 0.0388$ ,  $r = 0.4772$ ) and EPO ( $p < 0.0001$ ,  $r = 0.7807$ ) were positively correlated with baseline absolute eosinophil counts (AEC), there was no correlation between urine granule protein levels and AEC. Thirteen subjects had an AEC of 0 on benralizumab therapy at week 24. Despite a lack of peripheral eosinophilia, urine MBP ( $p = 0.0001$ ) and EPO ( $p < 0.0001$ ) levels were significantly increased compared to levels in healthy volunteers, whereas serum granule protein levels were at or below normal levels. Eight subjects had mild to moderate post-treatment reactions and elevated serum LDH following the first dose of benralizumab. Within 1 day of treatment, AEC and serum EPO were significantly decreased in subjects with ( $p = 0.0078$ ) and without ( $p = 0.0049$ ) post-treatment reactions. In contrast, urine EPO was significantly increased only in the subjects who experienced post-treatment reactions ( $p = 0.0078$ ).

**Conclusions:** Measurement of eosinophil granule proteins in urine is non-invasive and unaffected by cellular lysis during sample collection. Although levels of serum and urine eosinophil granule proteins showed a similar pattern following benralizumab treatment, the lack of correlation between urine granule protein levels and AEC and the observed association of urine EPO levels with post-treatment reactions suggest that urine measurement of granule proteins may provide a better biomarker of disease activity independent of AEC.

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39

### Fluid Shear Stress Induces Rapid Actin Rearrangement in Eosinophils

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**Background:** Complex interactions between eosinophil integrin receptors and their analogous ligands on the post-capillary venules of the bronchial endothelium result in temporally-regulated intracellular calcium [Ca<sup>2+</sup>]<sub>i</sub> signaling cascades and modifications to the cytoskeletal architecture. The current study investigates the effect of fluid shear stress as a stimulus for eosinophil morphological change and its relationship with the perfusion-induced calcium response (PICR).

**Methods:** Human eosinophils were isolated from peripheral blood of non-asthmatic donors using negative immunomagnetic selection. The cells were incubated with a calcium dye and plated onto fibronectin-coated coverslips within a parallel-plate flow chamber. Real-time confocal microscopy was utilized for fluorimetric and area tracings of individual cells to characterize the effects of physiologically-relevant rates of fluid shear stress on the [Ca<sup>2+</sup>]<sub>i</sub> and cytoskeleton regulating signaling pathways. In addition, time-based immunofluorescence staining of fixed eosinophils was employed to visualize the distribution of adhesion-related proteins at specific timepoints post-perfusion.

**Results:** Prior to perfusion, the cells were observed to be spherical with a smooth border, with minimal fluctuation in fluorescence signal. Approximately 90-200 seconds after the application of perfusion, the eosinophils experienced a sharp increase in [Ca<sup>2+</sup>]<sub>i</sub> followed by a less rapid return of fluorescence towards baseline. This release of calcium from intracellular stores coincided with morphological changes: a loss of circularity for cytoskeletal extensions, flattening onto the coverslip surface, and expansion in surface area. We observed that although the latency of the PICR post-perfusion was highly dependent on the magnitude of fluid shear stress, the nature and degree of the response remained fairly consistent across manipulations.

All immunofluorescence staining images obtained prior to perfusion show diffuse staining throughout the cell for our proteins of interest (activated  $\beta$ 1-integrin subunit; talin; vinculin), but filamentous actin (F-actin) showed stronger presence in the periphery. This marginal localization of F-actin became more pronounced after fluid shear stress application, ultimately clustering in distinct focal dots 5 minutes post-stimulation. The adhesion-related proteins were re-distributed towards the outer edges of the cell but exhibited minimal colocalization with F-actin as observed in merged fluorescence channels.

## Poster Abstract Session 1: Wednesday, 10 July 2019

**Conclusions:** We observed an all-or-nothing response with respect to release of calcium from intracellular stores and magnitude of morphological change, which aligns with our current understanding of cytoskeletal rearrangement, as partial responses with respect to shape change would be a misuse of energy. However, the time to response following shear stress stimulation showed a longer delay at the two extremes tested and a shorter delay at the intermediate degree of shear stress, which also corresponds to the level of shear stress found in post-capillary bronchial venules where eosinophils would normally migrate into the surrounding tissue. The present study illuminates the temporal link between cell flattening and the calcium spike, as well as their dependence on the degree of shear stress, and the potential roles of certain cytoskeletal components within this physiological response. We are interested in further exploring our finding that although morphological changes and calcium fluctuations are tightly linked temporally, they are not linked causally.

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41

### Krüppel-Like Factor 3 (KLF3) Regulates Adipose Tissue Beiging via Eosinophils

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Obesity is a global epidemic that is causing significant socioeconomic and health problems. An excess of white fat typifies obesity, but newly described beige fat can burn calories through its thermogenic activity, leading to weight loss and metabolic improvements. Identification of the factors regulating beige fat will guide the design of novel therapeutics to treat metabolic disorders.

We aimed to further characterise the lean, obesity-resistant phenotype of the KLF3 knockout mouse model by exploring whether the transcriptional repressor KLF3 plays a role in beige fat formation, i.e. the beiging process. Mice deficient in KLF3 have more beige fat than their wild-type littermates under room temperature housing conditions, evidenced by increased adipose tissue expression of thermogenic genes and proteins, such as uncoupling protein 1. Cold temperature is the classical stimulus for thermogenesis in beige fat cells, so we investigated the extent of beige fat activation by performing acute cold and thermoneutral temperature experiments with wild-type and KLF3 knockout mice. We found that KLF3 deficiency conferred a heightened response to the cold, with robust upregulation of beige fat and thermogenic genes compared to wild-type mice. The increased beiging in KLF3 knockout adipose tissue was also observed after thermoneutral housing, albeit to a lesser degree.

Previous work has shown that KLF3 does not play a direct role in regulating adipocyte transcription, therefore, we searched for other cell types involved in the beiging process that KLF3 might be important in. KLF3 is highly expressed in eosinophils, and interestingly, we found that mice lacking KLF3 have three times the number of eosinophils in their adipose tissue than wild-type mice. Recently, type 2 immune cells including eosinophils and alternatively activated macrophages have been shown to be an important part of an immune network which drives beige fat activation. Gene expression analyses have identified that KLF3 represses key genes in eosinophils, some of which have been shown to drive beige fat development. In the absence of KLF3, a suite of eosinophil genes is de-repressed which may allow eosinophils to secrete more of the factors that promote adipose tissue beiging, which we have termed 'eosinokines'. We aim to characterise the role of KLF3 in eosinophils in the context of adipose tissue beiging and to identify novel eosinokines using mice and cell models. This work will contribute to a growing understanding of the immune regulation of metabolism in order to help drive the development of targeted weight-loss therapeutics that activate beige fat.

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43

### Association of Blood Eosinophil Phenotypes and Patient Self-Assessment Data with Response to Treatment in Eosinophilic Esophagitis

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A threshold of 15 eosinophils per high power field in esophageal biopsies is used to determine if patients with eosinophilic esophagitis have responded to treatment, be it diet or topical corticosteroids. The main objectives of this study were to evaluate 1) if a blood-based panel of immune parameters focusing on eosinophil molecules could correctly identify response to treatment in eosinophilic esophagitis patients, and 2) if such a panel would mirror the patients' self-assessed symptomatic relief. Twenty adult patients newly diagnosed with eosinophilic esophagitis donated blood and filled in three questionnaires (Watson Dysphagia Scale, EORTC QLQ-OES18 and SF-36) before and after a 2-month course of topical corticosteroids. Blood samples were analyzed for levels of eosinophils, CD4 and CD8 T-cells, and

## Poster Abstract Session 1: Wednesday, 10 July 2019

the expression of 10 different eosinophil markers by 4-color flow cytometry. All data including questionnaire scores were evaluated by using multivariate analysis. The multivariate models revealed that patients who responded to treatment were older and had higher levels of eosinophils in the esophagus prior to treatment. Responders also had higher median fluorescence intensity of CD49d and CD193 on blood eosinophils pre-treatment, and a greater reduction in the percentage of CD16+ eosinophils after therapy. Additionally, improvements regarding local pain, cough, dysphagia and reflux symptoms, as well as the increased ability to eat meat and fruit were characteristics of the responders. The combination of blood-based immune parameters and patient-derived self-assessment data could be used to construct algorithms for monitoring of treatment response in the future, perhaps obviating the need for repeat biopsies.

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45

### Eosinophilic Gastrointestinal Disease Treatment Approaches are Associated with Frequency of Feelings of Isolation

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**Background:** Eosinophilic gastrointestinal diseases (EGIDs), including eosinophilic esophagitis (EoE), gastritis (EG), gastroenteritis (EGE), and colitis (EC), are associated with high patient burden. Using data collected from a contact registry (CR) of patients with EGIDs, established through collaboration between the Rare Disease Clinical Research Network, Consortium on Eosinophilic Gastrointestinal Disease Researchers (CEGIR) and its patient advocacy groups, we sought to evaluate the association between frequency of patient-reported feelings of isolation and EGID treatments. We hypothesized that treatment types associated with food restrictions would be associated with increased frequency of feelings of isolation.

**Methods:** We conducted a cross-sectional study evaluating EoE-associated treatments in relation to patient-reported feelings of isolation. We included CR participants who provided consent to share their demographic data and who completed a supplemental CR questionnaire. We identified participants reporting EoE only versus one or more other EGID condition. In bivariate analysis we compared the distribution of weekly or daily feelings of isolation, versus less frequent feelings of isolation (monthly, infrequently, or never) by treatment type. We stratified results by age (<18 years and 18 years or older). Differences in the distribution of frequency of feelings of isolation, by treatment type, within categories of EoE only versus another EGID, and within age groups, were made using the Chi-square test. Where data were sparse, the Fisher's exact test was used. Generalized linear models (binomial distribution, logit link) were used to estimate the crude and adjusted odds of weekly or daily feelings of isolation in relation to elemental formula, specific food elimination, and proton pump inhibitor (PPI) treatments, adjusting for food avoidance behaviors and frequency of diarrhea. We assessed whether odds of more frequent feelings of isolation in relation to treatment were modified by age. The study was approved by the University of South Florida Data Management Coordinating Center IRB.

**Results:** Of the 725 CR participants, 525 reported a diagnosis of EoE only and 210 reported one or more other EGIDs (with or without concomitant EoE) (n=10 missing an EGID type). Among adults with EoE only, a significantly higher proportion of those reporting weekly or daily feelings of isolation were treated with a specific food elimination approach as compared to those reporting less frequent feelings of isolation (80.6% versus 59.5%;  $p < 0.01$ ). For both pediatric and adult CR respondents, elemental diet therapy was associated with increased frequency of weekly or daily feelings of isolation ( $p = 0.04$  for <18 and  $p = 0.02$  for  $\geq 18$ ) (Table 1). For the other EGIDs, increased frequency of feelings of isolation was only associated with use of a PPI, and this was only among adults ( $p = 0.04$ ) (Table 2). After adjusting for food avoidance behaviors and frequency of diarrhea, specific food elimination and elemental formula treatments remained associated with an increased odds of more frequent feelings of isolation for participants with EoE only (adjusted OR: 2.4; 95% CI: 1.5, 4.1 for specific food elimination and adjusted OR: 1.9; 95% CI: 1.2, 3.3 for elemental formula) (Table 3). There was no evidence of modification by age in any of the adjusted analyses ( $p > 1.5$  for all).

# Poster Abstract Session 1: Wednesday, 10 July 2019

**Conclusions:** Disease-associated treatments, including the elemental diet and avoidance of certain foods, may contribute to increased frequency of feelings of isolation. Additional evaluation is needed, where treatment and patient reported outcomes have been documented longitudinally. If confirmed, these results may have implications on treatment decisions and recommendations for additional supportive measures to address psychosocial concerns for EGID patients.

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Table 1. Frequency of feelings of isolation by treatment and age for respondents with EoE only

| Treatment type              | Age <18                          |                       |       | Age ≥18                          |                       |       |
|-----------------------------|----------------------------------|-----------------------|-------|----------------------------------|-----------------------|-------|
|                             | Never, infreq., or monthly n (%) | Weekly or daily n (%) | p     | Never, infreq., or monthly n (%) | Weekly or daily n (%) | p     |
| PPI                         |                                  |                       |       |                                  |                       |       |
| No                          | 15 (15.0)                        | 11 (9.2)              | 0.19  | 23 (19.5)                        | 15 (16.0)             | 0.51  |
| Yes                         | 85 (85.0)                        | 108 (90.8)            |       | 95 (80.5)                        | 79 (84.0)             |       |
| Specific food elimination   |                                  |                       |       |                                  |                       |       |
| No                          | 14 (12.4)                        | 8 (6.5)               | 0.12  | 51 (40.5)                        | 19 (19.4)             | <0.01 |
| Yes                         | 99 (87.6)                        | 116 (93.6)            |       | 75 (59.5)                        | 79 (80.6)             |       |
| Topical/swallowed steroids  |                                  |                       |       |                                  |                       |       |
| No                          | 21 (21.2)                        | 31 (26.3)             | 0.38  | 35 (30.2)                        | 29 (31.5)             | 0.83  |
| Yes                         | 78 (78.8)                        | 87 (73.7)             |       | 81 (69.8)                        | 63 (68.5)             |       |
| Systemic steroids           |                                  |                       |       |                                  |                       |       |
| No                          | 74 (82.2)                        | 84 (73.7)             | 0.15  | 93 (86.1)                        | 68 (80.0)             | 0.26  |
| Yes                         | 16 (17.8)                        | 30 (26.3)             |       | 15 (13.9)                        | 17 (20.0)             |       |
| Mast cell stabilizer agents |                                  |                       |       |                                  |                       |       |
| No                          | 57 (66.3)                        | 68 (58.6)             | 0.27  | 77 (72.6)                        | 56 (63.6)             | 0.18  |
| Yes                         | 29 (33.7)                        | 48 (41.4)             |       | 29 (27.4)                        | 32 (36.4)             |       |
| Immunomodulatory agents     |                                  |                       |       |                                  |                       |       |
| No                          | 82 (98.8)                        | 106 (94.6)            | 0.24* | 95 (96.9)                        | 79 (96.3)             | 0.82* |
| Yes                         | 1 (1.2)                          | 6 (5.4)               |       | 3 (3.1)                          | 3 (3.7)               |       |
| Elemental diet              |                                  |                       |       |                                  |                       |       |
| No                          | 88 (76.5)                        | 81 (64.3)             | 0.04  | 123 (95.4)                       | 87 (87.0)             | 0.02  |
| Yes                         | 27 (23.5)                        | 45 (35.7)             |       | 6 (4.7)                          | 13 (13.0)             |       |

\*Fisher's Exact test

Table 2. Frequency of feelings of isolation by treatment and age for respondents with other EGIDs\*

| Treatment type             | Age <18                          |                       |        | Age ≥18                          |                       |      |
|----------------------------|----------------------------------|-----------------------|--------|----------------------------------|-----------------------|------|
|                            | Never, infreq., or monthly n (%) | Weekly or daily n (%) | p      | Never, infreq., or monthly n (%) | Weekly or daily n (%) | p    |
| PPI                        |                                  |                       |        |                                  |                       |      |
| No                         | 6 (28.6)                         | 10 (62.5)             | 0.53** | 14 (42.4)                        | 12 (21.8)             | 0.04 |
| Yes                        | 15 (71.4)                        | 41 (73.2)             |        | 19 (57.6)                        | 43 (78.2)             |      |
| Specific food elimination  |                                  |                       |        |                                  |                       |      |
| No                         | 5 (23.8)                         | 7 (12.3)              | 0.29** | 7 (20.6)                         | 8 (13.8)              | 0.39 |
| Yes                        | 16 (76.2)                        | 50 (87.7)             |        | 27 (79.4)                        | 50 (86.2)             |      |
| Topical/swallowed steroids |                                  |                       |        |                                  |                       |      |
| No                         | 8 (40.0)                         | 26 (53.1)             | 0.32   | 15 (50.0)                        | 25 (46.3)             | 0.74 |
| Yes                        | 12 (60.0)                        | 23 (46.9)             |        | 15 (50.0)                        | 29 (53.7)             |      |



# Poster Abstract Session 1: Wednesday, 10 July 2019

|                             |           |           |        |           |           |      |
|-----------------------------|-----------|-----------|--------|-----------|-----------|------|
| Systemic steroids           |           |           |        |           |           |      |
| No                          | 6 (30.0)  | 19 (38.0) | 0.53   | 9 (30.0)  | 15 (27.8) | 0.83 |
| Yes                         | 14 (70.0) | 31 (62.0) |        | 21 (70.0) | 39 (72.2) |      |
| Mast cell stabilizer agents |           |           |        |           |           |      |
| No                          | 11 (55.0) | 22 (46.8) | 0.54   | 13 (41.9) | 28 (51.9) | 0.38 |
| Yes                         | 9 (45.0)  | 25 (53.2) |        | 18 (58.1) | 26 (48.2) |      |
| Immunomodulatory agents     |           |           |        |           |           |      |
| No                          | 16 (88.9) | 40 (90.9) | 0.99** | 20 (76.9) | 41 (78.9) | 0.85 |
| Yes                         | 2 (11.1)  | 4 (9.1)   |        | 6 (23.1)  | 11 (21.2) |      |
| Elemental diet              |           |           |        |           |           |      |
| No                          | 13 (61.9) | 32 (56.1) | 0.65   | 27 (77.1) | 41 (70.7) | 0.50 |
| Yes                         | 8 (38.1)  | 25 (43.9) |        | 8 (22.9)  | 17 (29.3) |      |

\* EG, EGE, and EC with or without concomitant EoE

\*\*Fisher's Exact test

Table 3. Association between treatment and weekly or daily feelings of isolation, adjusting for symptoms and behaviors

| Treatment approach        | EoE only       |                        | EG, EGE, or EC* |                        |
|---------------------------|----------------|------------------------|-----------------|------------------------|
|                           | OR (95% CI)    | Adjusted OR** (95% CI) | OR (95% CI)     | Adjusted OR** (95% CI) |
| PPI                       |                |                        |                 |                        |
| No                        | referent       | referent               | referent        | referent               |
| Yes                       | 1.5 (0.9, 2.6) | 1.3 (0.8, 2.3)         | 2.1 (1.0, 4.4)  | 2.3 (1.0, 5.2)         |
| Specific food elimination |                |                        |                 |                        |
| No                        | referent       | referent               | referent        | referent               |
| Yes                       | 2.6 (1.6, 4.2) | 2.4 (1.5, 4.1)         | 1.9 (0.8, 4.4)  | 1.6 (0.6, 4.0)         |
| Elemental formula         |                |                        |                 |                        |
| No                        | referent       | referent               | referent        | referent               |
| Yes                       | 2.1 (1.3, 3.4) | 1.9 (1.2, 3.3)         | 1.5 (0.8, 3.1)  | 1.3 (0.6, 2.9)         |

\*EG, EGE, and EC with or without concomitant EoE

\*\*Adjusting for food avoidance behaviors and frequency of diarrhea or loose stools

## 47

### Human Eosinophils Express a Distinct Gene Expression Program in Response to IL-3 Compared to Common Beta-Chain Cytokines IL-5 and GM-CSF

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**Background:** Despite recent advances in asthma management with anti-IL-5 therapies, many patients with eosinophilic asthma remain poorly controlled. IL-3 shares a common beta subunit receptor with both IL-5 and GM-CSF, but through alpha subunit-specific properties, uniquely influences eosinophil biology and may serve as a potential therapeutic target. We aimed to globally characterize the transcriptomic profiles of GM-CSF, IL-3 and IL-5 stimulation and identify differences in gene expression using advanced statistical modeling.

**Methods:** Human eosinophils were isolated from the peripheral blood of healthy volunteers and stimulated with either GM-CSF, IL-3 or IL-5 for 48 hours. RNA was then extracted and bulk sequencing performed. DESeq2 (Bioconductor) analysis identified differentially expressed genes and weighted gene co-expression network analysis (WGCNA) independently defined modules of genes that are highly co-expressed.

**Results:** IL-3 stimulation yielded the most numbers of differentially expressed genes that were also highly co-expressed. WGCNA, which assigns genes into modules based on similar patterns of change in expression across samples, yielded further support for an IL-3-specific gene expression signal. Of the 24 modules identified by WGCNA, five modules were highly correlated with  $\beta$ -chain receptor cytokine stimulus groups ( $r > 0.5$ ,  $p < 0.01$ ). The modules with the two strongest correlations occurred in relation to IL-3. These two modules contained



## Poster Abstract Session 1: Wednesday, 10 July 2019

82% of the IL-3 upregulated genes identified by DESeq2 analysis, with most of these genes belonging to a single module. GM-CSF and IL-5 stimulation demonstrated redundancy in eosinophil gene expression.

**Conclusion:** IL-3 produces a distinct eosinophil gene expression program among the beta-chain receptor cytokines.

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49

### Eosinophils Suppress TH1 Responses and Restrict Bacterially Induced Gastrointestinal Inflammation

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Inflammatory diseases of the gastrointestinal (GI) tract are on the rise and are associated with high morbidity. There are substantial gaps in our understanding of their etiology and pathogenesis. Strong evidence suggests that GI eosinophils play pivotal roles in the pathogenesis of GI inflammatory conditions such as inflammatory bowel diseases (IBD) and *Helicobacter pylori*-induced gastritis. However, the function of eosinophils in these diseases is still poorly understood.

**Methods:** Here, we have examined the function and regulation of gastrointestinal eosinophils in the steady state and during infection with *Helicobacter pylori* or *Citrobacter rodentium*.

**Results:** We find that eosinophils are recruited to sites of infection, directly encounter live bacteria, and activate a signature transcriptional program; this applies also to human gastrointestinal eosinophils in humanized mice. The genetic or anti-IL-5-mediated depletion of eosinophils results in improved control of the infection, increased inflammation and more pronounced Th1 responses. Eosinophils control Th1 responses via the IFN- $\gamma$ -dependent upregulation of PD-L1. Furthermore, we find that the conditional loss of IFN- $\gamma$ R, but not of MyD88 signaling, phenocopies the effects of eosinophil depletion. Eosinophils further possess bactericidal properties that require degranulation and the deployment of extracellular traps.

**Conclusions:** Our results highlight two novel functions of this elusive cell type and link it to gastrointestinal homeostasis and anti-bacterial defense.

51

### Clinical and Endoscopic Features of the Fibrostenotic Phenotype in Eosinophilic Esophagitis

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**Background:** Phenotypes of eosinophilic esophagitis (EoE) are described in the literature including the inflammatory, fibrostenotic and mixed types. Current evidence suggests that the inflammatory phenotype is an earlier stage of the disease and without intervention will progress to the fibrostenotic type with the mixed type representing an intermediate stage. Whether these phenotypes have implications for disease activity, preferred treatment, or prognosis of disease is not known. We sought to determine whether the fibrostenotic phenotype of EoE exhibited any distinct clinical features.

**Methods:** In an IRB-approved protocol, 28 adults with eosinophilic esophagitis underwent endoscopy, with evaluation of the EoE Endoscopic Reference Score (EREFS) and EoE Histological Score (EoEHSS). All subjects also completed the Eosinophilic Esophagitis Activity Index Patient Reported Outcome (EEsAI PRO) questionnaire. In addition, whole blood was stained for eosinophil surface markers and analyzed by flow cytometry. The EREFS tool was used for determining phenotype, specifically the fibrostenotic phenotype was defined by a Ring score = 3 or any strictures. Clinical, histologic and demographic data were compared in the fibrostenotic versus non-fibrostenotic phenotypes.

**Results:** Patients were classified as fibrostenotic (FS, n=15) or non-fibrostenotic (NF, n=13). On biopsy, the mean peak eosinophil counts (PEC) seemed higher but not significantly in the FS vs. NF phenotypes (67.2 versus 48 eosinophils per high power field, p=0.14). Patients with the FS phenotype demonstrated a trend towards higher mean EoEHSS composite scores (13.3 versus 10.7, p=0.09) but there was no difference in EEsAI PRO questionnaire score (36.6 versus 30, p=0.42). Mean Fluorescence Intensity (MFI) of Alpha IIb Integrin, P-selectin, and N29 as assayed by flow cytometry were not different between the two phenotypes.

# Poster Abstract Session 1: Wednesday, 10 July 2019

**Conclusions:** Patients with the FS phenotype of EoE demonstrated a trend toward higher histology score, EoEHSS. Sample size likely limited our ability to detect clinically significant differences in the FS phenotype.

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53

## Molecular, Endoscopic, Histological and Circulating Biomarker-Based Diagnosis of Eosinophilic Gastritis: Cross Sectional Multi-Site Study

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**Background:** Eosinophilic gastritis (EG) is a clinico-pathological disorder with marked gastric eosinophilia and clinical symptoms. This study aimed to develop tissue and blood-based diagnostic platforms for EG, to validate their utility for diagnosis and management, and to better understand disease pathogenesis.

**Methods:** Patients with EG and non-EG controls were enrolled across 10 sites associated with the Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR). EG was diagnosed as gastric eosinophilia  $\geq 30$  eosinophils/HPF in  $\geq 5$  HPFs. Genome-wide gene expression profiles from gastric biopsies were generated by RNA sequencing. An EG Diagnostic Panel (EGDP) focusing on a set of 48 informative gastric transcripts and an EG blood biomarker panel based on a 10 protein multiplex array were analyzed for their performance in discovery and validation cohorts. The EGDP score was calculated by summation of delta CT values of the most highly dysregulated genes ( $\Sigma\Delta CT$ ). Blood EG scores were established by dysregulated cytokines/chemokine levels. For diagnosis, the area under the receiver operating characteristic curve (AUC) was calculated.

**Results:** Gastric biopsies (total n = 158; discovery n = 83 and validation n = 75) and blood samples (total n = 155; plasma n = 81 and serum n = 74) were analyzed. For the tissue-based platform, the EGDP score a) identified active EG patients ( $P < 0.0001$ ,  $AUC \geq 0.98$ ) in both cohorts; b) effectively monitored disease activity based on tissue eosinophil levels in longitudinally collected samples ( $P = 0.0078$ ); c) showed comparable levels and high correlation between the gastric antrum and body samples from the same patient ( $r = 0.90$ ,  $P < 0.0001$ ); d) demonstrated significant correlation with gastric peak eosinophil levels ( $r = -0.76$ ,  $P < 0.0001$ ), endoscopic severity ( $r = -0.54$ ,  $P < 0.0001$ ), histological glandulitis ( $r = -0.71$ ,  $P < 0.0001$ ) and endoscopic nodularity ( $r = -0.55$ ,  $P < 0.0001$ ). CCL26 was the most substantial gene associated with gastric eosinophilia, histological features, and endoscopic findings ( $P < 0.0001$ ). For blood-based platforms, the levels of 3 cytokines significantly increased ( $P < 0.05$ ) in both EG cohorts (plasma; CCL26, IL-5 and TARC, serum; TSLP, CCL26 and IL-5). Levels of these circulating cytokines a) distinguished EG patients from non-EG patients ( $P < 0.0001$ ,  $AUC \geq 0.94$ ); b) correlated with gastric eosinophil levels (plasma;  $r = 0.61$ ,  $P = 0.0008$ , serum;  $r = 0.66$ ,  $P = 0.0003$ ); c) correlated with the EGDP score (plasma;  $r = -0.52$ ,  $P = 0.0135$ , serum;  $r = -0.53$ ,  $P = 0.0017$ ); and was most notable for plasma CCL26 levels ( $r = -0.64$ ,  $P = 0.0006$ ).

**Conclusions:** We have developed tissue-based and circulating non-invasive biomarkers for EG. CCL26 has emerged as the strongest tissue and circulating disease biomarker. We have uncovered robust associations among the EG molecular profile, eosinophilic glandulitis, and endoscopic nodularity, providing insight into the better understanding of the pathogenesis for EG.

57

## Eosinophils Alter Steady-State Small Intestinal IL-1 $\alpha$ and IL-1 $\beta$ Levels but are not Required for Maintenance of Secretory IGA nor For Control of an Enteric Bacterial Pathogen

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**Background:** Eosinophils have been reported to play a role in controlling intestinal colonization by harmless or symbiotic members of the bacterial microbiota, however, to date, there have been no studies investigating their role during infection with bacterial pathogens.

56

## Poster Abstract Session 1: Wednesday, 10 July 2019

**Methods:** Wild-type BALB/c and eosinophil-deficient ( $\Delta$ dblGATA) mice were orally infected with the bacterial pathogen *Salmonella enterica* serovar Typhimurium. *Salmonella* burdens along the intestinal tract were quantified in addition to burdens in the liver and spleen to track levels of systemic infection. Intestinal inflammation was characterized by measuring both inflammatory and anti-inflammatory cytokine levels in both naive and infected mice using cytometric bead array assays. An ELISA was utilized to quantify secretory immunoglobulin A (sIgA) in naive wild-type and eosinophil-deficient mice, both before and after co-housing mice of different genotypes to control for potential microbiota diversities.

**Results:** We found that levels of IL-1 $\alpha$  and IL-1 $\beta$  were significantly decreased in the small intestine of naive eosinophil-deficient mice, compared to wild-type mice. Despite this reduction in inflammatory cytokines, following *Salmonella* infection, *Salmonella* burdens were similar between wildtype and eosinophil-deficient mice both 24 hours and three days post-infection. Additionally, contrary to previously published reports, we did not detect any deficiency in sIgA levels in naive eosinophil-deficient mice, neither prior to, nor following cohousing with wild-type mice.

**Conclusion:** Our data supports a role for eosinophils in modifying steady-state cytokine levels in the intestinal tract. However, in our *Salmonella* infection model, an absence of eosinophils did not impact control of *Salmonella* infection. Our data further suggest that eosinophils are not critical for the maintenance of intestinal sIgA.

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59

### Transgenic Expression of a Novel Secreted Active Form of IL-33 Promotes Eosinophilia in a Mouse Model of Eosinophilic Esophagitis

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**Background:** IL-33 is an inflammatory cytokine that induces eosinophils and other cells to produce Th2 cytokines such as IL-13. IL-33 is produced in an inactive form and stored in the cell nucleus presenting hurdles for transgenic studies of IL-33 activity. We hypothesized that transgenic expression of a secreted and active form of IL-33 would overcome these hurdles and promote eosinophilic inflammation.

**Methods:** We generated a secreted active mouse IL-33 fusion gene by combining the IL-2 secretory signal peptide gene sequence with that encoding an active IL-33 fragment. Fusion gene function was examined *in vitro* with a tetracycline-inducible system transfected into HEK-293 cells. IL-33 production was assessed by immunohistochemistry (IHC). Fusion gene activity was examined by culturing eosinophils in the HEK-293 cell culture supernatants and assessing IL-13 production by ELISA. Fusion gene activity was examined *in vivo* by transgenic expression from the esophageal epithelium. Mice were subjected to OVA sensitization/gastric challenge protocol. Eosinophilia was assessed by H&E and eosinophil peroxidase IHC.

**Results:** Fusion gene transfected HEK-293 cells produced IL-33. Eosinophils produced IL-13 in response to the fusion gene product. Expression of the fusion gene by the esophageal epithelium resulted in esophageal eosinophilia at baseline that was increased relative to Wild Type in an eosinophilic esophagitis model.

**Conclusions:** Fusion of the IL-2 secretory signal peptide sequence with that encoding an active IL-33 fragment generated secreted and active IL-33. Expression of the IL-33 fusion gene from the esophageal epithelium promoted esophageal eosinophilia. The ability to express secreted and active IL-33 will facilitate studies examining activities of IL-33.

61

### Hypereosinophilia Associated with Clonal CD3-CD4+ T Cells: Characterization and Outcome of a New Cohort of Patients

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**Background:** Lymphocytic variant hypereosinophilic syndrome (L-HES) is a well-established disease, characterized by the presence of clonal CD3-CD4+ T cells driving eosinophil expansion and activation through secretion of interleukin (IL)-5. Being an orphan disorder, it has proven difficult to delineate disease presentation and outcome, and treatment responses.

## Poster Abstract Session 1: Wednesday, 10 July 2019

**Methods:** A retrospective in-depth characterization of a mono-centric cohort of subjects with clonal CD3-CD4+ T cells and blood and/or tissue hypereosinophilia. Our cohort was compared to the recently published French national cohort using appropriate statistical tests.

**Results:** Twenty-four subjects with circulating CD3-CD4+ T cells and associated hypereosinophilia were included. Mean age at first symptoms and discovery of HE was 40 years, and the M/F ratio was 11/13. All patients were symptomatic except one, classified as HE<sub>US</sub>. The most frequent symptoms/signs were skin lesions (87%), angioedema (52%), lymphadenopathy (56%), muscle pain and induration (30%), and pulmonary disease (17%). None of the patients had cardiac involvement.

Mean circulating eosinophil counts were 4.65 G/L at diagnosis, peaking above 10 G/L in 42% of cases. Lymphocytosis was observed in only 2 subjects. Serum IgE levels were increased in 54%, IgG and/or IgM in 50%, and LDH in 64%. The mean percentage of blood CD3-CD4+ T cells was 18.7% (range 0.15-71%), with less than 2% abnormal cells detected in 8 cases. A clonal TCR gene rearrangement pattern was found on whole blood in 50% of patients.

Maintenance treatment of symptomatic patients consisted in prolonged oral corticosteroid (CS) monotherapy in 6 cases, and second-line CS-sparing agents in 9 patients who all required >10 mg PDN daily for disease control. The remaining 8 subjects, who had significantly lower CD3-CD4+ counts, had mild disease and did not require maintenance therapy. In all, 15 patients received oral CS monotherapy for at least 6 months; none were CS-resistant, and the mean threshold of CS-dependency was 19.2 mg PDN (range 5-50). Treatment responses could be evaluated in 5/8 and 3/5 patients treated with IFN-alpha and hydroxyurea respectively. One patient experienced disease cure (disappearance of CD3-CD4+ cells, eosinophilia, and symptoms) with combined IFN-alpha and CS, and the 4 other subjects had a partial clinical and biological response but treatment was interrupted due to toxicity and/or lack-of-efficacy. Only one patient experienced a partial response to hydroxyurea, but the dosing required was associated with intolerable toxicity. Other agents including cyclosporin-A, cyclophosphamide, IFN-gamma, and imatinib, were ineffective. Fludarabine was administered to one patient with progressive disease, resulting in a complete but temporary response.

Disease course was heterogeneous, with 3 patients experiencing spontaneous improvement at one end of the spectrum, and 7 patients with rapidly progressive disease requiring aggressive management at the other. Among the latter, there were 3 deaths and 2 malignant progressions. In all, 3 patients developed T cell lymphoma, 2 of whom died of complications of chemotherapy, while one failed to respond and was subsequently cured with ASCT. Over time, CD3-CD4+ T cell counts were stable or decreased with treatment in all subjects except one. No relationship was observed between abnormal T cell counts and malignant progression.

No statistically significant differences were observed between the French cohort and ours in terms of clinical and biological characteristics.

**Conclusions:** The predominant cutaneous and soft tissue manifestations in L-HES are confirmed in this large mono-centric cohort. However, disease course and treatment responses are heterogeneous. Progressive and severe disease is observed in roughly one third of patients, who often remain symptomatic despite therapeutic escalation with high-dose oral CS and second-line agents. Together with the French cohort, the incidence of lymphoma can be accurately estimated at 11% (5/47). There is a strong need for identification of novel therapeutic targets for progressive treatment-refractory disease and lymphoma.

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### 63

#### CD200R1 is Upregulated in the Esophageal Epithelium in Eosinophilic Esophagitis

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**Background:** Eosinophilic esophagitis (EoE) is an emerging type 2 inflammatory disease that has unique histopathology. These pathological changes include extensive tissue remodeling such as epithelial hyperplasia, epithelial mesenchymal transition (EMT), impaired mucosal integrity, eosinophilic inflammation, collagen deposition and tissue fibrosis that directly associates with disease manifestations. Our group recently demonstrated that it is possible to divide EoE patients into subgroups according to their clinical, pathological and molecular changes that may reflect the disease progression (e.g. EoE endotypes 1-3). However, pathways that are capable of counter-regulating disease progression been underexplored. CD200R1 is a cell surface immune inhibitory receptor involved in leukocyte activation, differentiation and proliferation. Herein, we examine CD200R1 and its ligand CD200 expression in EoE, aiming to elucidate its potential functional contribution to EoE.

**Material and Methods:** To define the cellular source accounting for esophageal CD200R1 expression, polychromatic flow cytometric staining was conducted using single cell suspensions obtained from the esophageal biopsies. Biopsies from EoE patients and normal controls were subjected to immunohistochemistry staining (IHC). In order to define the role of CD200R1 in esophageal epithelial cell, primary epithelial cell were activated using the ligand CD200Fc and were examined by proliferation assays (MTT, Ki67 and Phospho-H3).

## Poster Abstract Session 1: Wednesday, 10 July 2019

**Results:** CD200R1 mRNA expression was 20-fold higher ( $P < 0.0001$ ) in biopsies from EoE patients compared to control individuals. In addition, the expression of CD200R1 protein was increased in esophageal biopsies obtained from EoE patients. The highest expression was in the subgroup of patients characterized with the inflammatory endotype (EoE2). CD200R1 was highly expressed by esophageal epithelial cells and inflammatory leukocytes, predominantly by mast cells. However epithelial cells were the only cells that significantly up-regulated CD200R1 expression in EoE patients compared to control individuals. In contrast, the increased expression of CD200R1 by mast cells was associated with the increase in this cell population in EoE. Preliminary analysis of the CD200R1 activation by its ligand, CD200, showed that CD200-FC inhibited the proliferation of CD200R1+ primary epithelial cells (30% inhibition;  $p < 0.05$ ).

**Conclusion:** These data demonstrates that epithelial cells in EoE biopsies have increased expression of the CD200R1. Evidence is presented that this is an inhibitory pathway on esophageal epithelium, and may thus be a key regulatory pathway and therapeutic entry point for EoE.

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65

### Eosinophil Subtype Specific Metabolic Activities

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**Rationale:** Eosinophils are modulated by the local cytokine milieu of the tissue microenvironment in both homeostatic conditions and in disease progression with the function of regulating Local Immunity and/or Remodeling/Repair (LIAR hypothesis). Recently, exposure of eosinophils to type 1 or type 2 cytokines has demonstrated subtype specific effector functions. Eosinophil subtypes are characterized by expression of specific genes and we hypothesize that their functions are supported by a distinct metabolic phenotype. The goal of this study was to examine relationships between type 1 and type 2 cytokine-dependent gene expression and parameters of energy metabolism in mouse eosinophils.

**Methods:** Pure blood-derived mouse eosinophils were cultured with cytokines (IL-33, GM-CSF, and IL-4 (type 2) or TNF-A and IFN-g (type 1)) typical for type 1 or type 2 environment to polarize the cells to their subtypes (E2 and E1) or without these cytokines (E0). RNA was extracted for RNA-seq transcriptome analysis and rates of oxygen consumption and extracellular acidification were directly assessed in polarized eosinophils using a Seahorse XFe96 Analyzer.

**Results:** RNA-seq analysis showed elevated expression of genes present in mitochondrial respiratory chain in Th2 compared to Th1 cytokine treated eosinophils. Consistent with these observations E2 eosinophils had significantly higher maximal respiratory capacity and rate of media acidification compared to E1 eosinophils.

**Conclusion:** Our studies showed an overall elevated metabolic rate in type 2 cytokine treated eosinophils (E2 eosinophils), suggesting a link between eosinophil subtypes and parameters of their energy metabolism.

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67

### Eosinophils Modulate ILC2 Activities

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**Rationale:** Allergic respiratory inflammation is characterized by type 2 immune responses that include infiltration of innate cells that propagate the inflammatory response. Classically, eosinophilic infiltration is measured as an indicator of disease severity or as a marker of response to therapeutic intervention. As such, recent advances in FDA approved therapeutics are aimed at eosinophil ablation as a means of attenuating disease. Yet, the recent discovery of group 2 innate lymphoid cells (ILC2s) has led to a new focus and a paradigm shift that



# Poster Abstract Session 1: Wednesday, 10 July 2019

ILC2s are key upstream mediators of eosinophil recruitment and activation. Our studies indicate, rather, that eosinophils and ILC2s interact in a complex node of reciprocal interactions in type 2 responses.

**Methods:** Type 2 pulmonary inflammation was induced by either intratracheal cytokine administration (e.g., IL-33) or using models of ovalbumin or house dust mite allergen sensitization/challenge. Eosinophils were specifically depleted immediately prior to instillation or challenge using inducible eosinophil-deficient mice (iPHIL) mice to determine the role of eosinophils on pulmonary ILC2s in these type 2 inflammation models. Lung-derived ILC2s were cultured with eosinophils to define ILC2 and eosinophil interactions. Activation and chemotaxis of ILC2s were assessed *in vitro*.

**Results:** Our studies presented here demonstrate eosinophils are important in modulating the activities of ILC2s in the early cascade of type 2 inflammatory responses. Using mouse models of inducible eosinophil deficiency (iPHIL) we demonstrated a significant reduction in ILC2 numbers and activation state in several mouse models of type 2 pulmonary inflammation. Thus the method and/or route of inducing type 2 pulmonary inflammation is insignificant compared to the absence or presence of eosinophils in participating in the accumulation of IL-13+/IL-5+ ILC2s within the lung compartment.

**Conclusion:** Together these data suggest a reciprocal role for eosinophil-ILC2 interactions in amplification of the type 2 pulmonary inflammatory response.

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69

## Inhibition of Eosinophil Transendothelial Migration by 5HTP Stimulation of HTR1A and HTR1B in Endothelial Cells

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

**Objective:** We hypothesize that dietary supplementation with the amino acid 5-HTP reduces eosinophil recruitment and allergic inflammation by acting on the serotonin receptors HTR1A and HTR1B in endothelial cells.

**Methods:** Serotonin receptor expression. HTR expression in endothelial cells and eosinophils was determined by western blot and qPCR. Inhibition of Serotonin Receptors. Selective pharmacological inhibitors of HTR1A, HTR1B were used. In addition, serotonin receptor expression was blocked with siRNA during bone marrow differentiation of eosinophils or in endothelial cell lines. Expression was determined by western blot. *In vitro* eosinophil transendothelial migration assays with laminar flow. Endothelial cell monolayers were treated with 5HTP overnight and washed. Eosinophil transendothelial migration at 15 minutes was examined under conditions of laminar flow of 2 dynes/cm<sup>2</sup>.

**Results:** The serotonin receptors HTR1A, HTR1B and HTR3A are expressed by endothelial cells and eosinophils as determined by western blot and qPCR. Selective HTR inhibitors and siRNA for HTR1A and 1B in endothelial cells blocked 5HTP inhibition of migration. These data indicate that these endothelial serotonin receptors are involved in 5HTP inhibition of transendothelial migration and thus may serve as a target for intervention during allergic inflammation. These results will help design ongoing clinical studies addressing whether 5HTP supplementation reduces allergic asthmatic responses.

**Conclusions:** In summary, dietary supplementation with the readily available amino acid 5-hydroxytryptophan (5HTP) inhibits eosinophil recruitment to the lung and inhibits leukocyte transendothelial migration *in vitro* by stimulating endothelial cell serotonin receptors HTR1A and HTR1B. This novel approach for regulation of eosinophilic inflammation identifies potential targets for intervention in allergic inflammation.

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## PIN1 Regulates IL-5 Induced Eosinophil Polarization and Migration

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**Background:** IL-5 family cytokines induce eosinophil polarization and nucleopod formation which require F-actin reorganization and microtubule dynamics. The morphological changes likely facilitate vascular arrest, migration, degranulation and possibly the survival of eosinophils in inflamed tissues. Rho family GTPases are essential for F-actin polymerization and hence cell motility. Pin1, a *cis-trans* peptidyl-prolyl isomerase (PPIase), regulates eosinophil survival and migration through interactions with a subset of signaling molecules that contain phosphorylated Ser-Pro or Thr-Pro motifs (pS/pT-Pro). Once bound, Pin1 isomerizes the pS/pT-Pro peptide bond to *cis/trans* conformers, thereby altering target protein structure, function and catabolism. Pin1 isomerase activity is rapidly increased by pro-survival cytokines (IL-5 and GM-CSF) *in vitro* as well as *in vivo* after allergic challenge.

**Methods:** Human eosinophils were purified from blood and Pin1 WT and knockout mouse eosinophils were differentiated *in vitro* from bone marrow. Cells were starved for 1 h before treatment with IL-5 for 10 min or 18 h with IL5/eotaxin. Human EOS were pretreated with Pin1 inhibitor peptides (TAT-WW) or control peptides (TAT-W34A). The effects of Pin1 blockade on cellular phenotypes were analyzed by phase contrast microscopy (cell length/width), Boyden chamber (migration), immunoblots, qPCR, immunoprecipitation and immunostaining.

**Results:** Mouse eosinophils lacking Pin1 or human cells treated with Pin1 inhibitors were resistant to IL-5-induced shape changes, F-actin polymerization and directional cell migration towards chemokines. The IL-5-induced cellular phenotypes were similarly blocked when human eosinophils were pre-incubated with Rac2 or RhoA inhibitors. Immunoblots and qPCR analysis revealed lower endogenous levels of Rho-family GTPases (Rac2, RhoA and Cdc42) in Pin1 KO eosinophils compared to WT. Co-immunoprecipitation (IP) and immunofluorescence cell staining demonstrated that Pin1 directly interacts with the Rho GTPases and that, in response to IL-5, Pin1 itself re-localizes to the nucleopod (enriched in PSGL1, CD44, integrins and IL-5 receptor) from the cytosol.

**Conclusions:** Pin1 regulates eosinophil morphology, cytoskeletal re-organization and cell migration through modulation of Rho GTPases activity and gene expression.

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## Adipose Tissue Eosinophils in Cancer-Associated Cachexia

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**Background:** Cancer associated cachexia is a syndrome characterized by pathologic loss of adipose tissue (AT) which contributes significantly to the morbidity and mortality of many malignancies. Tumor-derived inflammatory factors such as IL-6 and TNF $\alpha$  have been implicated as drivers of cachexia, but whether AT-resident immune cells contribute to this pathology is unknown.

**Methods:** We are using spectral flow cytometry, immunohistochemistry, RNA-sequencing, and quantitative proteomics to analyze eosinophils in adipose tissue.

**Results:** We identified a population of SSC<sup>hi</sup>SiglecF<sup>+</sup>Gr1<sup>-</sup> cells comprising up to 70% of the myeloid fraction in the gonadal and inguinal white adipose tissue (gWAT, iWAT) of healthy mice. H&E staining revealed a pure eosinophil population; further, this population is absent from the AT of  $\Delta$ dbIGATA mice. Analysis of publicly available single cell RNA-sequencing of murine AT suggests that adipose mesenchymal stem cells are likely important in the recruitment of eosinophils via production of eotaxin-1.

Although eosinophil frequencies are similar between healthy and cachectic CT26 (colon) tumor-bearing mice, we hypothesize that functional differences in eosinophils may contribute to pathology. Considering previous reports on the role of eosinophils as drivers of white AT being and the depletion of AT eosinophils during exposure to high fat diet, we hypothesize that eosinophils can be functionally redirected to drive cachexia-associated fat loss. To evaluate this hypothesis, we are performing RNA-sequencing and quantitative proteomics of sorted AT eosinophils under healthy and cachectic conditions. To establish this protocol, we first characterized eosinophils sorted from the spleen and bone marrow, where we have observed high expression of eosinophil-defining transcription factors and enzymes at the tran-

# Poster Abstract Session 1: Wednesday, 10 July 2019

script levels. We further report proteomic analysis of 2200 identified proteins from mouse eosinophils and compare to published datasets from similarly evaluated human eosinophils.

**Conclusions:** Eosinophils comprise a majority of the adipose tissue hematopoietic compartment. This population does not change in number during the progression of cancer associated cachexia in the models that we have studied to date, but we hypothesize that AT eosinophils undergo functional changes which contribute to the development of cachexia. RNA-sequencing and quantitative proteomics will allow us to assess such functional changes robustly in an unbiased manner.

75

## Pharmacodynamic and Clinical Efficacy Data from Patient Sputum Subgroups in Dream Treated With Mepolizumab across a 10-Fold Dose Range

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**Background:** Sputum eosinophil data from ‘all-comers’ in the phase 3 DREAM study showed a pharmacodynamic dose-response across a 10-fold mepolizumab dose range, which appeared to be of limited clinical relevance as clinical efficacy was comparable, as measured through exacerbation rate reduction. There are now several approved therapies targeting eosinophilic inflammation; however, the role of sputum eosinophils as a biomarker for assessing dose response is unclear. The objective of this analysis was to explore the pharmacodynamic and clinical effect of mepolizumab IV across a 10-fold dose range in subpopulations with either sputum eosinophilia  $\geq 3\%$  or with oral corticosteroid (OCS) dependent asthma.

**Methods:** DREAM (N=621) was a 52-week dose-ranging phase 2b/3 study of mepolizumab IV in severe asthma patients. A subset (n=94) from selected sites provided sputum samples during the study (baseline, week 4, week 16, week 52, and follow-up). Sputum samples were processed within 2 hours of collection and sputum cell count analysis was conducted at a centralized site. In this post hoc analysis, we examined sputum eosinophil and exacerbation rate reduction across a 10-fold mepolizumab dose range.

**Results:** At baseline, 70% (n=66) of ‘all comers’ in the sputum group had sputum eosinophilia  $\geq 3\%$  with a mean eosinophil count of 19.18%. When compared to baseline there was an 84% and 96% reduction in sputum eosinophils with 75mg IV and 750mg IV mepolizumab, respectively, compared to a 47% reduction with placebo (Table 1). When compared to placebo the exacerbation rate reduction was 71% (95% CI 28-89%) and 69% (95% CI 27-87%), with 75mg IV and 750mg IV mepolizumab, respectively. Forty-three percent (n=40) of ‘all comers’ in the sputum group were OCS-dependent with a mean sputum eosinophil count of 9.63%. In this group, there was a 70% and 94% reduction in sputum eosinophils with 75mg IV and 750mg IV mepolizumab, respectively, compared to a 55% reduction with placebo (Table 1), translating into an exacerbation rate reduction compared to placebo of 59% (95% CI 2-82%) and 80% (95% CI 48-92%), respectively.

**Conclusions:** Treatment with mepolizumab across a 10-fold dose range resulted in a dose dependent sputum eosinophil reduction in patients with  $\geq 3\%$  sputum eosinophils, and in those who were OCS-dependent. The pharmacodynamic dose response appears to be more pronounced in the OCS-dependent subpopulation with a smaller reduction in sputum eosinophils following mepolizumab 75mg IV. Despite this, the reduction in exacerbation rate compared to placebo was clinically relevant in both subpopulations with a reduction of 59% or more across the 10-fold dose range providing direct evidence that a dose response on sputum eosinophils is not predictive of treatment response.

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**Table 1. DREAM Subpopulations: Sputum Eosinophil Reduction with Mepolizumab**

|   | Placebo<br>N=27 | Mepolizumab<br>75 mg IV<br>N=20 | Mepolizumab<br>250 mg IV<br>N=24 | Mepolizumab<br>750 mg IV<br>N=23 |
|---|-----------------|---------------------------------|----------------------------------|----------------------------------|
| <b>Sputum eosinophils <math>\geq 3\%</math> subpopulation</b> |                 |                                 |                                  |                                  |
| n [1]   | 16              | 15                              | 20                               | 15                               |
| <b>Baseline</b>   |                 |                                 |                                  |                                  |
| n   | 16              | 15                              | 20                               | 15                               |

# Poster Abstract Session 1: Wednesday, 10 July 2019

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|--|---------------|---------------|---------------|---------------|
| Sputum eosinophils (%)*                                | 22.28 (0.980) | 23.55 (0.868) | 14.58 (0.938) | 19.20 (0.838) |
| <b>Week 52</b>   |               |               |               |               |
| n  | 10            | 8             | 14            | 9             |
| Sputum eosinophils (%)*                                | 6.95 (1.942)  | 4.74 (2.565)  | 1.19 (1.691)  | 0.50 (1.889)  |
| n  | 10            | 8             | 14            | 9             |
| Ratio to baseline*                                     | 0.53 (2.253)  | 0.16 (2.049)  | 0.07 (2.043)  | 0.04 (2.313)  |
| <b>Oral corticosteroid-dependent sputum population</b> |               |               |               |               |
| n[1]   | 10            | 13            | 11            | 11            |
| <b>Baseline</b>  |               |               |               |               |
| n  | 9             | 11            | 10            | 10            |
| Sputum eosinophils (%)*                                | 9.80 (1.862)  | 16.49 (1.404) | 5.55 (2.226)  | 9.10 (1.794)  |
| <b>Week 52</b>   |               |               |               |               |
| n  | 6             | 9             | 8             | 7             |
| Sputum eosinophils (%)*                                | 3.00 (2.447)  | 4.23 (2.431)  | 3.67 (0.931)  | 0.91 (1.840)  |
| n  | 6             | 8             | 7             | 6             |
| Ratio to baseline*                                     | 0.45 (2.907)  | 0.30 (2.221)  | 0.52 (2.357)  | 0.06 (2.757)  |

n [1] – number of subjects in subpopulation

\*Geometric mean (SD logs), data were log transformed prior to analysis

77

## Palmitoylation of Galectin-10 in Human Eosinophils

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**Background:** In human eosinophils, galectin-10 is the most prominent constituent comprising an estimated 7% to 10% of total cellular protein, and forms Charcot-Leyden crystals (CLC); yet its regulation and function have remained undefined. We investigated CLC palmitoylation, which is one of most important reversible post-translational modifications and mediators of protein function.

**Methods:** To test whether CLC undergoes palmitoylation, we used two different assays: 1) the acyl-PEG exchange (APE) and 2) azide-alkyne click chemistry. The APE assay uses N-ethylmaleimide (NEM) to block free cysteines followed by hydroxylamine treatment to remove thioester-linked fatty acid groups attached to cysteine residues, which are then reacted with methoxy-PEG-maleimide (mPEG-Mal) of defined mass and analyzed by Western blot. The click chemistry method uses palmitic acid azide to metabolically label palmitoylated proteins within eosinophils, followed by CLC immunoprecipitation, copper initiated click reaction with biotin-alkyne. Biotinylated palmitoylation of CLC is then detected with HRP-streptavidin.

**Results:** Using the APE assay, which can detect hydroxylamine cleavable fatty acid thioesters, likely palmitic acid, we demonstrated that endogenous CLC proteins were palmitoylated in freshly isolated human eosinophils. To further confirm that specific palmitoylation of CLC occurs within human eosinophils, incorporation of palmitic acid azide was evaluated by click chemistry mediated assays. We found that palmitic acid was metabolically incorporated into CLC proteins. Both experimental approaches demonstrate that CLC protein is palmitoylated in human eosinophils.

**Conclusions:** CLC protein was previously shown to cleave palmitic acid from lysopalmitoyl phosphatidylcholine. Our current findings in human eosinophils demonstrate that CLC proteins undergo palmitoylation within eosinophils. Palmitoylated CLC protein might function as a palmitoyltransferase and may facilitate interactions with other sub plasmalemma proteins, potentially involved in secretion.

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## Poster Abstract Session 2: Thursday, 11 July 2019

2

### Eosinophils from Normal Donors: How Do They Vary? How Do They Define Us?

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**Background:** Eosinophils contain numerous preformed immunomodulatory mediators including cytokines and chemokines which are contained in cytoplasmic granules and are released to the local microenvironment in response to specific activating agents. The extent to which peripheral blood vary from one individual to another has not been examined extensively or systematically.

**Methods:** EDTA-anticoagulated whole blood (30 mL) from normal adult human donors was obtained following informed consent via the Laboratory of Allergic Diseases normal volunteer protocol (09-I-0049) or the NIH Clinical Center Blood Bank. Eosinophils were isolated by negative selection (MACSxpress, Miltenyi) and lysates were prepared (20 mM tris pH 8.0, 139 mM NaCl, 0.5 μM EDTA, 1% Igepal and 10% glycerol with 1 tablet protease inhibitor mix (Roche #04693159001) per 10 mL lysis buffer) at 10<sup>7</sup> cells / mL. Eosinophil lysates were prepared and used to screen for the relative abundance of cytokines using the human cytokine array profiler (ARY005B; R & D Systems) with one donor selected to serve as normalization standard throughout. Critical results were confirmed and extended by ELISA DuoSet (R&D Systems).

**Results:** The donors included 18 males (42 ± 16 years old) and 14 females (38 ± 17 years old), all with hematologic parameters within normal range. Eosinophils (> 98% purity and viability) were isolated from an initial set of fourteen (14) initial donors. From this primary evaluation, we identified three groups of cytokines: eighteen (18) cytokines that varied minimally among donors (average variation <1.25-fold), eleven (11) cytokines that varied moderately among donors (average variation 1.55- to 1.97-fold), and one cytokine that varied dramatically. In the latter group, eosinophil concentrations of the alarmin-stressorin, interleukin-16, varied over a ~40-fold range among the initial donors (average variation 6.08 ± 6.3), and in an extended donor pool (n = 32), absolute concentrations of immunoreactive IL-16 ranged from 115 to greater than 13,000 pg / mg lysate protein. Most intriguing, eosinophil IL-16 concentrations (pg / mg) did not correlate with age or with any hematologic parameter, but did correlate directly with BMI (body mass index) in a specific subset of donors (R<sup>2</sup> = 0.70, \*\*\*p < 0.0001)

**Conclusions:** Peripheral blood eosinophils from normal donors are not uniform. New methods for purification and screening permit us to evaluate peripheral blood eosinophils and their components both efficiently and quantitatively. We have found that eosinophils contain varying quantities of the cytokine, interleukin-16. Absolute levels of IL-16 vary dramatically among donors at levels that correlate directly with individual BMIs in a specific subset of normal donors.

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4

### Determining the Role of Eotaxin-1 in Eosinophil Development in Order to Promote Heterogeneity via an *Ex Vivo* Culture System

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**Background:** Eosinophils are proinflammatory granulocytes that play prominent roles in asthma as well as skin, gastrointestinal, and respiratory allergies. As such, an improved understanding of the basic biology of eosinophils is critical for the development of rational immunomodulatory therapeutics to combat these disorders. Eotaxin-1 is understood primarily as the major eosinophil chemoattractant, but our preliminary data show that it may have significant impact on eosinophil differentiation. In this study, we explore the role of murine eotaxin-1 in eosinophil development and differentiation from the stem cell stage to a fully differentiated and functionally active granulocyte.

**Methods:** Bone marrow-derived eosinophils (bmEos) were generated as per standard methods. Murine bone marrow was extracted from wild-type BALB/c mice and cultured in media containing 100 ng/mL SCF (stem cell factor) and Flt-3l (FMS-like tyrosine kinase 3 ligand) for 4 days allowing for the selective expansion of hematopoietic stem cells. On Day 4, the cells were washed and added to media containing IL-5, with the exception that bmEos were grown in 0.5 ng/mL rather than 10 ng/mL IL-5; we also included 500 pg/mL CCL11 (eotaxin-1). Eosinophils were evaluated by flow cytometry, Proteome Profiler and ELISA, and qPCR. Functional properties were determined using chemotaxis and survival assays.

**Results:** Most eosinophils circulating in the periphery are characterized as SiglecF<sup>+</sup>/Gr1<sup>-/low</sup>. However, when analyzing the mature bmEos cultured *ex vivo*, we detect an 80/20 mix of Gr1<sup>+/high</sup> to Gr1<sup>-/low</sup>. These data indicate that, while IL-5 promotes eosinophil development in allergic states, the specific concentration of IL-5 may be critical with respect to eosinophils heterogeneity and gene expression. Notably, as

## Poster Abstract Session 2: Thursday, 11 July 2019

serum IL-5 levels are low at homeostasis, our findings suggest that other factors, including cytokines or chemokines, may promote eosinophil development. At homeostasis, eotaxin-1 (CCL11) is detected in both serum and bone marrow of wild-type BALB/c mice at concentrations of approximately 500 pg/mL and 35 pg/mL, respectively. First, we determined that the IL-5 concentration in primary culture could be reduced from 10 ng/mL to 0.5 ng/mL with no significant loss in eosinophil production or viability. Furthermore, when bmEos were grown in 0.5 ng/mL of IL-5 with 500 pg/mL CCL11, the resulting culture contained a significantly larger fraction of SiglecF<sup>+</sup> Gr1<sup>-low</sup> bmEos (43%) which is closer to what is observed physiologically than what emerged from the established 10 ng/mL IL-5 culture method (18%). Further analysis showed that CD125 and CD34 expression was significantly increased in the presence of eotaxin-1, but not in response to the lower concentration of IL-5 (0.5 ng/mL) alone. As eotaxin-1 has such a significant impact on the phenotype of bmEos, it raises the question as to whether other eosinophil chemoattractants including MIP-1alpha (CCL3) and/or RANTES (CCL5) may also play a role in development in addition to their roles in eosinophil activation.

**Conclusions:** Our data suggest that eotaxin-1 has a significant impact on the structural and functional characteristics of eosinophils cultured *ex vivo*, suggesting that this mediator may play a crucial role in their development.

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### 6

#### Eosinophilia and Hookworm Infection: A Living Drug for Autoimmune Diseases

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**Background.** Epidemiological studies show inverse relationships between MS severity and infections with gut helminths. MS patients naturally infected with gut helminths have a reduction in symptoms and MRI lesions, associated with an increase in regulatory T cells (Treg) and a shift to Th2-type immune responses. Eosinophilia was a biomarker of infection and protection against the development of MS. (Signature eosinophilia @  $1.4 \times 10^9/L$ )<sup>1,2</sup>. In supportive studies, controlled infection with 10-50 infective *Necator americanus* (hookworm, HW) larvae was shown to be safe, resulting in a significant eosinophilia, indicating a Th2 shift. 25 larvae provided a good balance between immune modulation and tolerability.

**Methods.** We performed a phase II, randomized, double-blind, placebo controlled trial of *N americanus* in relapsing MS. Inclusion criteria were relapsing MS patients off immunotherapy, MRI consistent with MS, one relapse in the prior 12 months or 2 in the prior 24 months, EDSS 0-5.5 at screening, and the ability to provide informed consent. The primary outcome measure was the cumulative number of new and enlarging T2 lesions and gadolinium enhancing lesions at month 9 post infection. Secondary outcomes included Treg numbers, relapse rate, and EDSS change at month 9. A run-in MRI was performed at month 3, then monthly for 6 months. Anthelmintic mebendazole was prescribed to all patients at month 9.

**Results.** There were no baseline differences between the two arms in the number of T2 lesions or EDSS. At month 9, T2 lesion number was not different between the arms as assessed by a pre-established Mann-Whitney test ( $p=0.15$ ). However, the distribution of new lesion numbers rendered the Mann-Whitney test inappropriate, due to a disproportionate number of patients in the HW group without new lesions ( $p=0.04$  by Fisher's exact test). Treg numbers increased in the hookworm group ( $p=0.013$ ), and potential benefit was associated with an eosinophilia exceeding that seen in naturally protected populations. There were 10 relapses in the placebo group as opposed to 4 in the HW group.

**Conclusions.** Hookworm treatment was well tolerated and showed indications of a therapeutic immune modulatory effect, as reported in naturally infected populations. An opportunity exists to expand studies of this type, to include safe, long-lived (15 yrs) and tolerable booster infections, in an attempt to strengthen the immune modulatory impact of *Necator americanus*. Its potential as an easily delivered long-term living drug should be explored further. This conclusion is supported by the successful application of hookworm therapy in coeliac disease.

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# Poster Abstract Session 2: Thursday, 11 July 2019

8

## Sustained Long-Term Efficacy and Safety of RPC4046, an Anti-Interleukin-13 Monoclonal Antibody, in Patients with Eosinophilic Esophagitis: Results from the Open-Label Extension of the Heroes Study

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**Background:** HEROES (NCT02098473) was a 16-week, double-blind (DB), placebo-controlled, phase 2, multicenter study followed by a 52-week open-label extension (OLE) in adults with active eosinophilic esophagitis (EoE). In the DB period, RPC4046 demonstrated a statistically significant reduction in esophageal eosinophil count and improvements in EoE Endoscopic Reference Score (EREFS), EoE Histology Scoring Symptom (EoEHSS) grade and stage score, and patient perception of disease severity and symptoms, with an acceptable safety profile.

**Methods:** Patients who completed the 16-week DB, placebo-controlled period entered the OLE and received weekly RPC4046 360 mg subcutaneously. Esophageal biopsies and clinical assessments occurred at OLE weeks 12, 24, and 52. Outcomes included assessment of mean and peak esophageal eosinophil count, EoEHSS, EREFS, symptoms (Eosinophilic Esophagitis Symptom Activity Index [EEsAI]), and safety. Esophageal eosinophil counts and histology score were determined by a central pathologist. OLE analysis was performed according to the original DB treatment assignment.

**Results:** In the DB period, 99 patients were randomized 1:1:1 to receive RPC4046 180 mg (n=31), RPC4046 360 mg (n=34), or placebo (n=34). Of 90 subjects completing the 16-week DB period, 86 entered the OLE and 66 completed an additional 52 weeks of therapy. Mean esophageal eosinophil counts (cells/high-powered field) in the OLE remained stable in patients on RPC4046 360 mg or 180 mg prior to the OLE but improved rapidly in patients on placebo prior to the OLE (Table). Similar effects by treatment group prior to OLE entry were seen across other outcome measures including peak eosinophil count, EREFS over all locations, and grade and stage EoEHSS (Table). During the OLE, the proportion of patients achieving symptomatic remission as determined by an EEsAI score ≤20 was 24.4% (OLE baseline), 44.4% (week 12), 51.3% (week 24), and 58.2% (week 52). The most frequent adverse events (AEs) (≥10%) during the OLE were upper respiratory tract infection, nasopharyngitis, oropharyngeal pain, sinusitis, and headache, and the types and incidence rates of AEs in the OLE were consistent with those in the DB period. The overall incidence rate of AEs/100 patient-years of exposure remained consistent in the OLE relative to the DB period.

**Conclusions:** Patients who received RPC4046 180 mg or 360 mg in the DB period and received 360 mg in the OLE had sustained clinical and histologic improvement of EoE disease activity through 52 weeks. Patients who received placebo during the DB period and then received RPC4046 360 mg in the OLE showed improvement by week 12 that was maintained through week 52. Generally, the overall incidence of AEs remained consistent with increasing exposure, with the types of AEs reported during the OLE similar to those reported during the DB period. Longer-term treatment with RPC4046 was generally safe and well tolerated with no safety concerns through 52 weeks.

**Mean Esophageal Eosinophil Counts by Visit in the Open-Label Extension (RPC4046 360 mg)**

| Visit                        | Double-Blind Randomized Treatment Group |                       |                       | Total (N=86) |
|------------------------------|---|-----------------------|-----------------------|--------------|
|                              | Placebo (n=29)                          | RPC4046 180 mg (n=28) | RPC4046 360 mg (n=29) |              |
| Esophageal Eosinophil Counts |   |                       |                       |              |
| OLE Baseline, mean (SD)      | 88.4 (55.9)                             | 27.1 (36.9)           | 25.6 (30.5)           | 47.3 (51.4)  |
| OLE Week 12, mean (SD)       | 21.2 (21.4)                             | 21.9 (32.3)           | 35.2 (43.2)           | 26.0 (33.6)  |
| OLE Week 24, mean (SD)       | 20.4 (21.0)                             | 24.5 (24.1)           | 21.1 (24.3)           | 22.0 (22.9)  |
| OLE Week 52, mean (SD)       | 20.7 (26.8)                             | 15.1 (23.1)           | 24.8 (38.1)           | 20.1 (29.8)  |
| EREFS Total Score            |   |                       |                       |              |
| OLE Baseline, mean (SD)      | 8.1 (5.1)                               | 5.5 (3.8)             | 6.5 (4.4)             | 6.7 (4.6)    |
| OLE Week 12, mean (SD)       | 5.0 (4.1)                               | 4.3 (3.9)             | 4.0 (3.8)             | 4.4 (3.9)    |
| OLE Week 52, mean (SD)       | 3.0 (3.1)                               | 4.6 (4.4)             | 3.0 (2.4)             | 3.6 (3.5)    |

## Poster Abstract Session 2: Thursday, 11 July 2019

|                                |             |             |             |             |
|--------------------------------|-------------|-------------|-------------|-------------|
| EoEHSS – Histology Grade Score |             |             |             |             |
| OLE Baseline, mean (SD)        | 40.9 (13.5) | 21.5 (12.4) | 20.0 (6.5)  | 27.5 (14.7) |
| OLE Week 12, mean (SD)         | 19.3 (6.7)  | 19.5 (10.6) | 22.5 (9.4)  | 20.4 (9.0)  |
| OLE Week 52, mean (SD)         | 19.9 (9.0)  | 19.5 (9.2)  | 21.9 (11.6) | 20.4 (9.9)  |
| EoEHSS – Histology Stage Score |             |             |             |             |
| OLE Baseline, mean (SD)        | 40.9 (12.7) | 21.7 (12.6) | 19.4 (7.0)  | 27.4 (14.7) |
| OLE Week 12, mean (SD)         | 18.9 (7.5)  | 19.2 (11.0) | 21.8 (10.1) | 20.0 (9.6)  |
| OLE Week 52, mean (SD)         | 20.4 (9.9)  | 21.5 (10.2) | 22.2 (11.2) | 21.4 (10.3) |
| SD, standard deviation         |             |             |             |             |

### 10

#### Markers of Esophageal Epithelial-Mesenchymal Transition (EMT) are Significantly Reduced in Active Eosinophilic Esophagitis Following 16 Weeks of Treatment with RPC4046, an Anti-Interleukin-13 Monoclonal Antibody

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**Background:** HEROES (NCT02098473) was a 16-week, double-blind, placebo-controlled, phase 2, multicenter study that evaluated the efficacy and safety of RPC4046 in adults with active eosinophilic esophagitis (EoE). The study demonstrated a statistically significant reduction in esophageal eosinophil count and improvement in histopathologic parameters with RPC4046 treatment. Fibrostenosis of the esophagus is a known complication of EoE and may be mediated in part by epithelial-mesenchymal transition (EMT). We sought to determine whether treatment with RPC4046 modulates EMT.

**Methods:** Esophageal biopsy sections were taken at baseline and week 16 from 69 patients randomly assigned to weekly subcutaneous (SC) RPC4046 180 mg (n=19), 360 mg (n=26), or placebo (n=24). We stained slides by duplex immunofluorescence for e-cadherin and vimentin (Cell Signaling Technologies), counterstained nuclei with DAPI, and scanned at 20x on a Vectra® (PerkinElmer) multispectral digital microscopy system. Using inForm® software, a machine learning algorithm mapped the epithelial compartment in each slide. We defined nuclear, cytoplasmic, and membrane areas of each epithelial cell and recorded fluorescence intensity of each marker on a per-cell basis. For this EMT substudy, the primary endpoint was change from baseline in percentage of vimentin-positive epithelial cells and secondary endpoints were change in total e-cadherin expression per cell (total intensity of e-cadherin signal in membrane) and change in vimentin:e-cadherin ratio per cell. Over 6.45 million cells (average of 47,000 epithelial cells per biopsy) were analyzed.

**Results:** Change from baseline in the mean percentage of vimentin-positive cells was -2.75%, -4.24%, and -0.94% in the RPC4046 180 mg, 360 mg, and placebo groups, respectively ( $P < 0.05$  for 360 mg vs placebo). Change in mean e-cadherin expression per cell was 102.4, 101.6, and 18.3 in the RPC4046 180 mg, 360 mg, and placebo groups ( $P < 0.05$  for each active dose group vs placebo). The change in vimentin:e-cadherin ratio was significantly different from zero in both dose groups (180 mg, -0.18; 360 mg, -0.30;  $P < 0.05$  for each active dose group vs zero). Similar effects for all markers were observed in each esophageal sampled region (proximal, mid, distal).

**Conclusions:** RPC4046 treatment for 16 weeks significantly improved EMT markers in esophageal tissue in patients with active EoE. A greater effect generally was observed with 360 mg than with 180 mg. These results, together with the overall clinical data presented separately, support the hypothesis that prevention of IL-13 binding to receptor subtypes IL-13R $\alpha$ 1 and IL-13R $\alpha$ 2 favorably impacts inflammatory and remodeling pathways and may reduce the development of esophageal fibrostenotic complications that occur in EoE. Larger studies with longer-term treatment are required to determine the impact of these results on the course of EoE.

## Poster Abstract Session 2: Thursday, 11 July 2019

12

### Differentiation and Activation of Eosinophils in the Human Bone Marrow at Steady State and During Experimental Acute Systemic Inflammation

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**Background and objective:** Little is known on eosinophil differentiation and maturation in situ in human bone marrow in homeostasis and in response to acute inflammation. Acute systemic inflammation such as evoked by experimental endotoxemia leads to a rapid and transient eosinopenia. It is not clear whether this is caused by decreased mobilization/production of eosinophils in the bone marrow (BM) or merely a result of increased homing from the blood to the tissues. The objective of this study was to investigate the differentiation, maturation and activation status of eosinophils in the BM and blood during homeostasis and during an acute innate immune challenge in humans.

**Methods:** BM and blood were obtained from 12 healthy male volunteers in the age range between 20 and 30 years old before and during experimental human endotoxemia (a model of systemic inflammation evoked by intravenous challenge with 2 ng/kg endotoxin). Promyelocytes, myelocytes, metamyelocytes and mature eosinophils were identified and FACS sorted according to their CD11b/CD62L expression. Maturation and activation status were assessed using antibodies against known membrane markers on eosinophils: LAIR1 (CD305), Alpha-4 (CD49d), CCR3 (CD193), FcγRI (CD64), CR1 (CD35), CEACAM-8 (CD66b), IL-5Rα (CD125) and IL-3Rα (CD123). In addition, cytokines were analyzed in plasma and BM supernatant under both conditions.

**Results:** Eosinophil promyelocytes were characterized by a unique phenotype of surface markers: CD193<sup>dim</sup>, CD64<sup>dim</sup>, CD11b<sup>-</sup> and CD62L<sup>-</sup>. During maturation into myelocytes the cells changed to a CD193<sup>dim</sup>, CD64<sup>-</sup>, CD11b<sup>bright</sup> and CD62L<sup>-</sup> profile. Metamyelocytes were characterized as CD193<sup>bright</sup>, CD64<sup>-</sup>, CD11b<sup>bright</sup> and CD62L<sup>dim</sup> cells. Finally, mature eosinophils displayed CD193<sup>bright</sup>, CD64<sup>-</sup>, CD11b<sup>bright</sup> and CD62L<sup>bright</sup> expression.

Four hours after endotoxin administration, the percentage of mature eosinophils in the blood and in the BM declined (from  $3.0 \pm 1.9\%$  to  $0.2 \pm 0.11\%$  and  $1.3\% \pm 0.5\%$  to  $0.7 \pm 0.47\%$  respectively) whereas the number of eosinophil progenitors did not change. The remaining eosinophils in the circulation failed to show signs of activation or degranulation despite significantly increased circulating eotaxin-1 levels after LPS administration ( $p = 0.02$ ). However, the expression of CD49d and IL-5Rα on circulatory eosinophils was lower after LPS administration compared to baseline (both  $p = 0.03$ ).

**Conclusion:** The maturation of eosinophils in the bone marrow can be followed by staining cells in the granulocyte gate with a limited number of antibodies: CD64, CD193, CD11b and CD62L. A systemic innate immune challenge caused mature eosinophil mobilization from the BM without obvious signs of activation nor accelerated maturation of their progenitors. Circulatory eosinopenia evoked by systemic innate immune response is most likely the result of (CD49d) mediated homing to the tissues.

**Grant Support:** None.

14

### Activated Eosinophils Promote an Anti-Tumorigenic Environment in Tumor Models of Colorectal Cancer

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**Background:** Tumor-associated tissue eosinophilia has been described in various solid tumors. However, whether eosinophils play a role in tumor promotion or suppression is not clear. In colorectal cancer, data suggest that tissue eosinophilia accounts for a positive outcome in patients. Nevertheless, the mechanism behind a possible tumor-rejecting effect by eosinophils is not yet well understood. We aimed to elucidate the role of eosinophils on tumor growth in *in vitro* assays and in a murine tumor model with colorectal cancer (CRC) cells.

**Methods:** In order to investigate the role of eosinophils in colon cancer *in vivo*, two different *in vivo* treatment models of subcutaneously engrafted syngeneic CRC cell lines were used in Balb/c and eosinophil-deficient dβGATA-1. In one setting, endogenous eosinophils were activated and in the second setting, mice were administered cultured activated eosinophils intravenously. Mice were sacrificed three weeks after tumor challenge and ex vivo tumor weight was measured, followed by digestion and flow cytometric analysis of infiltrating immune cell populations.

**Results:** Isolated murine eosinophils showed specific migration towards CRC cell line-conditioned supernatants *in vitro*. Furthermore, intravenous injection of eosinophils into mice with subcutaneous tumors led to their recruitment to the tumor site within 6 hours.

## Poster Abstract Session 2: Thursday, 11 July 2019

Activated eosinophils in CRC cell-engrafted mice showed significantly smaller tumors in both models. Flow cytometric analysis revealed altered abundance of lymphocyte content in the tumor microenvironment of eosinophil-injected mice compared to untreated littermates. The cytotoxicity of eosinophils against tumor cells was confirmed *in vitro*, by showing that co-incubation of eosinophils with CRC cells led to a decrease in tumor cell viability after 24 hours.

**Conclusions:** Eosinophils are important effector cells that specifically migrate towards CRC *in vitro* as well as *in vivo* to mediate cytotoxicity. Furthermore, treatment with eosinophils shifted the tumor microenvironment to an anti-tumorigenic state resulting in reduced tumor size in mice. Taken together, these data suggest that eosinophils play an anti-tumorigenic role in syngeneic CRC cell line tumor models.

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16

### Vitamin Deficiencies May Vary in Relation to Symptoms and Treatment Approaches for Eosinophilic Esophagitis

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**Background:** As part of the Rare Disease Clinical Research Network (RDCRN), the Consortium of Eosinophilic Gastrointestinal Researchers (CEGIR), together with associated Patient Advocacy Groups (PAGs), established an Eosinophilic Gastrointestinal Disease (EGID) Contact Registry (CR). CR participants are provided the opportunity to complete an on-line supplemental questionnaire, documenting patient-reported experience as it relates to their diagnosis. Surprisingly, a high proportion of CR participants with EoE report having experienced vitamin deficiencies. Using data collected from the CR, we sought to evaluate the association between self-reported vitamin deficiencies and patient-reported gastrointestinal symptoms and treatments. We hypothesized that more frequent symptoms and treatments associated with dietary restrictions would be associated with self-reported vitamin deficiencies.

**Methods:** We conducted a cross-sectional study evaluating gastrointestinal symptoms, and EoE-associated treatments in relation to patient-reported vitamin deficiencies. We included CR participants who provided consent to share their demographic data and who completed a supplemental CR questionnaire. We identified participants reporting EoE only (versus one or more other EGID condition). We compared the distribution of gastrointestinal symptoms and treatment types by participant-reported vitamin deficiencies. Statistical comparisons were made using the Chi-square test. Where data were sparse, the Fisher's Exact test was used. Generalized linear models (binomial distribution, logit link) were used to estimate the crude and adjusted odds of self-reported vitamin deficiency in relation to treatment, adjusting for food avoidance behaviors and frequency of diarrhea. The study was approved by the University of South Florida IRB.

**Results:** Of the 725 CR participants, 525 reported a diagnosis of EoE only. A higher proportion of CR participants reporting vitamin deficiencies also reported avoidance of foods due to symptoms ( $p=0.01$ ) (Table 1). CR participants reporting vitamin deficiencies also reported more frequent symptoms of upper and lower abdominal pain, bloating, constipation, diarrhea, ( $p<0.01$  for all) and weight loss ( $p=0.01$ ). CR respondents treated with a Proton Pump Inhibitor (PPI) ( $p=0.01$ ), specific food elimination ( $p=0.02$ ), and/or systemic steroids ( $p=0.02$ ) also more frequently self-reported vitamin deficiencies (Table 2). After adjusting for food avoidance behaviors and frequency of diarrhea, PPI usage remained significantly associated with self-reported vitamin deficiencies (adjusted OR: 2.3; 95% CI: 1.2, 4.7) (Table 3).

**Conclusions:** Frequency of disease symptoms and disease-associated treatments may be associated with vitamin deficiencies. Additional evaluation is needed, perhaps in blood samples obtained and where treatment and disease symptoms have been documented longitudinally. If these results are confirmed, clinical assessments of vitamin deficiencies may be warranted in patient with EoE, especially those with high degree of symptoms and PPI usage.

## Poster Abstract Session 2: Thursday, 11 July 2019

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Table 1. Patient factors and symptoms and self/proxy-reported vitamin deficiency for patients with EoE only (n=525)

| Patient factor/symptom             | Vitamin deficiencies |              | p     |
|------------------------------------|----------------------|--------------|-------|
|                                    | No*<br>n (%)         | Yes<br>n (%) |       |
| Age                                | 193 (51.3)           | 86 (58.5)    | 0.14  |
| <18                                | 183 (48.7)           | 61 (41.5)    |       |
| Avoidance of foods due to symptoms | 94 (24.9)            | 20 (13.6)    | 0.01  |
| *No                                | 284 (75.1)           | 127 (86.4)   |       |
| Yes                                |                      |              |       |
| Vomiting or regurgitation          | 82 (23.6)            | 21 (14.4)    | 0.12  |
| Never                              | 95 (27.4)            | 38 (26.0)    |       |
| Infrequently/Quarterly             | 68 (19.6)            | 37 (25.3)    |       |
| Monthly                            | 68 (19.6)            | 30 (20.6)    |       |
| Weekly                             | 34 (9.8)             | 20 (13.7)    |       |
| Daily                              |                      |              |       |
| Chest pain                         | 113 (33.2)           | 35 (24.1)    | 0.39  |
| Never                              | 62 (18.2)            | 31 (21.4)    |       |
| Infrequently/Quarterly             | 66 (19.4)            | 31 (21.4)    |       |
| Monthly                            | 67 (19.7)            | 31 (21.4)    |       |
| Weekly                             | 32 (9.4)             | 17 (11.7)    |       |
| Daily                              |                      |              |       |
| Upper abdominal pain               | 79 (23.1)            | 15 (10.2)    | <0.01 |
| Never                              | 49 (14.3)            | 28 (19.1)    |       |
| Infrequently/Quarterly             | 67 (19.6)            | 28 (19.1)    |       |
| Monthly                            | 94 (27.5)            | 36 (24.5)    |       |
| Weekly                             | 53 (15.5)            | 40 (27.2)    |       |
| Daily                              |                      |              |       |
| Lower abdominal pain               | 119 (34.8)           | 24 (16.4)    | <0.01 |
| Never                              | 59 (17.3)            | 27 (18.5)    |       |
| Infrequently/Quarterly             | 65 (19.0)            | 28 (19.2)    |       |
| Monthly                            | 62 (18.1)            | 38 (26.0)    |       |
| Weekly                             | 37 (10.8)            | 29 (19.9)    |       |
| Daily                              |                      |              |       |
| Bloating                           | 129 (38.2)           | 34 (23.3)    | <0.01 |
| Never                              | 65 (19.2)            | 25 (17.1)    |       |
| Infrequently/Quarterly             | 54 (16.0)            | 24 (16.4)    |       |
| Monthly                            | 54 (16.0)            | 31 (21.2)    |       |
| Weekly                             | 36 (10.7)            | 32 (21.9)    |       |
| Daily                              |                      |              |       |
| Constipation                       | 137 (40.3)           |              | <0.01 |
| Never                              | 72 (21.2)            | 39 (26.9)    |       |
| Infrequently/Quarterly             | 43 (12.7)            | 23 (15.9)    |       |
| Monthly                            | 51 (15.0)            | 29 (20.0)    |       |
| Weekly                             | 37 (10.9)            | 34 (23.5)    |       |
| Daily                              |                      | 20 (13.8)    |       |



## Poster Abstract Session 2: Thursday, 11 July 2019

|                          |            |            |       |
|--------------------------|------------|------------|-------|
| Diarrhea or loose stools | 122 (35.8) |            |       |
| Never                    | 69 (20.2)  | 32 (22.1)  | <0.01 |
| Infrequently/Quarterly   | 67 (19.7)  | 28 (19.3)  |       |
| Monthly                  | 62 (18.2)  | 18 (12.4)  |       |
| Weekly                   | 21 (6.2)   | 44 (30.3)  |       |
| Daily                    |            | 23 (15.9)  |       |
| Blood in stools          | 269 (80.8) |            |       |
| No                       | 64 (19.2)  | 101 (73.2) | 0.07  |
| Yes                      |            | 37 (26.8)  |       |
| Weight loss              | 188 (55.6) | 60 (41.7)  | 0.01  |
| No                       | 150 (44.4) | 84 (58.3)  |       |
| Yes                      |            |            |       |

\*Assumes non-affirmative response is a “no” response

Table 2. Treatment and self/proxy-reported vitamin deficiency for patients with EoE only (n=525)

| Treatment type*                     | Vitamin deficiencies |              | p    |
|-------------------------------------|----------------------|--------------|------|
|                                     | No**<br>n (%)        | Yes<br>n (%) |      |
| PPI (n=383)                         |                      |              | 0.01 |
| No                                  | 57 (18.0)            | 11 (8.2)     |      |
| Yes                                 | 259 (82.0)           | 124 (91.9)   |      |
| Specific food elimination (n=387)   |                      |              | 0.02 |
| No                                  | 76 (22.4)            | 19 (13.4)    |      |
| Yes                                 | 264 (77.7)           | 123 (86.6)   |      |
| Topical/swallowed steroids (n=319)  |                      |              | 0.15 |
| No                                  | 92 (30.1)            | 32 (23.4)    |      |
| Yes                                 | 214 (69.9)           | 105 (76.6)   |      |
| Systemic steroids (n=80)            |                      |              | 0.02 |
| No                                  | 237 (83.8)           | 96 (73.9)    |      |
| Yes                                 | 46 (16.3)            | 34 (26.2)    |      |
| Mast cell stabilizer agents (n=146) |                      |              | 0.11 |
| No                                  | 190 (67.1)           | 76 (58.9)    |      |
| Yes                                 | 93 (32.9)            | 53 (41.1)    |      |
| Immunomodulatory agents (n=15)      |                      |              | 0.48 |
| No                                  | 258 (96.6)           | 118 (95.2)   |      |
| Yes                                 | 9 (3.4)              | 6 (4.8)      |      |
| Elemental diet (n=93)               |                      |              | 0.06 |
| No                                  | 287 (83.2)           | 111 (76.0)   |      |
| Yes                                 | 58 (16.8)            | 35 (24.0)    |      |

\*Treatment types are not unique

\*\*Assumes non-affirmative response is a “no” response

Table 3. Treatment and vitamin deficiencies, adjusting for symptoms in EoE

| Treatment                 | OR (95% CI)    | Adjusted OR* (95% CI) |
|---------------------------|----------------|-----------------------|
| PPI                       |                |                       |
| No                        | Referent       | Referent              |
| Yes                       | 2.5 (1.3, 5.0) | 2.3 (1.2, 4.7)        |
| Specific food elimination |                |                       |
| No                        | Referent       | Referent              |
| Yes                       | 1.9 (1.1, 3.2) | 1.7 (1.0, 3.0)        |

## Poster Abstract Session 2: Thursday, 11 July 2019

|                   |                |                |
|-------------------|----------------|----------------|
| Topical steroids  |                |                |
| No                | Referent       | Referent       |
| Yes               | 1.4 (0.9, 2.2) | 1.3 (0.8, 2.1) |
| Systemic steroids |                |                |
| No                | Referent       | Referent       |
| Yes               | 1.8 (1.1, 3.1) | 1.6 (0.9, 2.7) |
| Elemental diet    |                |                |
| No                | Referent       | Referent       |
| Yes               | 1.6 (1.0, 2.6) | 1.4 (0.9, 2.4) |

\*Adjusting for food avoidance behaviors and frequency of diarrhea or loose stools

### 18

#### Epidermal Expression of Eotaxins-1, 2, and 3 in Bullous Pemphigoid is Comparable to Other Eosinophil Rich Dermatoses: A Retrospective Immunohistochemical Analysis

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**Background:** Bullous pemphigoid is characterized by an eosinophil-rich superficial dermal infiltrate, with infiltration of eosinophils into the epidermis. We aimed to better understand eotaxin expression in bullous pemphigoid compared to other eosinophil rich dermatoses.

**Methods:** we performed a retrospective study of 49 biopsy specimens taken from patients with either immunoserologically confirmed bullous pemphigoid (n=15), atopic dermatitis (n=12), drug eruption (n=8), arthropod assault (n=5), and non-bullous pemphigoid eosinophilic spongiosis (n=5). Lichen planus (n=4) was used as a negative control given the lack of eosinophilia in the inflammatory infiltrate. Two investigators independently assessed eotaxin staining in the basal keratinocyte layer and spinous keratinocyte layer. The gradient between basal and spinous keratinocyte expressed was calculated.

**Results:** We used a Kruskal-Wallis test to compare eotaxin expression between each diagnostic group, as well as to compare the gradient of expression between basal keratinocytes and spinous keratinocytes. Our analysis failed to identify any significant discrepancies in eotaxin expression amongst eosinophil rich dermatoses.

**Conclusions:** Eotaxin expression appears to be a non-specific finding in inflammatory dermatoses with or without a large eosinophil infiltrate. There is gradient of eotaxin expression between basal keratinocytes and spinous keratinocytes. More sensitive techniques are needed to validate these comparative findings.

**Grant Support:** This project was supported in part by a grant from the International Pemphigus and Pemphigoid Foundation (KTA).

### 20

#### Detection of Eosinophil Cytolytic Degranulation in Paraffin-Embedded Tissue

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**Background:** Cytolytic degranulation, which has been studied using transmission electron microscopy, is a major mode of eosinophil degranulation in various inflamed tissues. Recent data indicated that cytolytic degranulation was mediated by an active form of cell death, termed extracellular trap cell death (ETosis). To assess cytolytic degranulation in tissues is critical for studying eosinophil activation and cytotoxicity; however, the detection of cytolytic degranulation in paraffin-embedded tissues has not been established.

**Methods:** We used immunofluorescence staining with anti-MBP and anti-galectin-10 antibodies. Cellular localization of MBP and galectin-10 during cytolysis was studied in vitro using isolated blood eosinophils stimulated with PMA and immobilized IgG. Paraffin-embedded tissue samples (nasal polyps, skin, and lung) were obtained from patients with eosinophilic chronic rhinosinusitis and eosinophilic granulomatosis with polyangiitis.

**Results:** In live eosinophils, galectin-10 and MBP staining was present in the cytoplasm and granules, respectively. In ETotic eosinophils, cytoplasmic galectin-10 was lost, although MBP was retained in intact granules. In tissue samples, intact eosinophils were stained with

## Poster Abstract Session 2: Thursday, 11 July 2019

galectin-10 and MBP, although lytic cells were only positive for MBP. Galectin-10 and MBP positive areas in fluorescence microscopy images were quantified using ImageJ software.

**Conclusions:** These results indicate that intact and lytic eosinophils in tissues can be differentiated using galectin-10 and MBP antibody staining. Galectin-10 and MBP staining might be a marker for tissue eosinophil degranulation.

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22

### Human Eosinophils Express Of Free Fatty Acid Receptor 2 And 3: Possible Implication of These Receptors in Interleukin-4 Production by Short Chain Fatty Acids

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**Background:** Short chain fatty acids (SCFAs), acetate, propionate, and butyrate, are produced by gut microbiota as fermentation products and transported to other tissues via the blood circulation. These SCFA molecules activate G protein-coupled receptors

(GPCRs), free fatty acid receptor (FFAR) 2 and 3, to be implicated in a variety of immunological and metabolic responses. Our previous transcriptomic analysis showed that FFAR2 and FFAR3 are highly upregulated at the terminal stage of eosinophilic differentiation. In the present study, we investigate expression of FFAR2 and FFAR3 and their possible function in response to SCFAs in cord blood (CB)-derived and peripheral blood (PB) eosinophils.

**Methods:** CB eosinophils are separated from human CB using CD34 microbeads and cultured using a cytokine cocktail IL-3, GM-CSF, and IL-5. PB eosinophils are isolated by using negative selection with CD16 microbeads. Surface FFAR2 and FFAR3 were measured by flow cytometry. Secreted and non-secreted forms of IL-4 were analyzed by anti-IL-4 antibodies, APC-MP4 and PE-8D4, respectively. IL-4 levels in culture supernatant were measured by ELISA. Acetylation of histone H3 was probed by anti-H3K27Ac.

**Results:** FFAR2 and FFAR3 were increasingly expressed as CD34+ cells isolated from

CB were induced to differentiate toward eosinophilic lineage, and also expressed in PB eosinophils, with greater expression of FFAR3 over FFAR2 in eosinophils from both sources. When CB and PB eosinophils were treated with butyrate, but not acetate or propionate, they expressed intracellular IL-4 in dose- and time-dependent manners with negligible IFN- $\gamma$ . The majority of the intracellular IL-4 turned out to be a non-secreted form. Nonetheless, PB eosinophils secreted a small amount of IL-4 at less than 1 pg/mL per  $1 \times 10^6$  cells. The butyrate-mediated IL-4 production did not appear to be associated with the inhibition of histone deacetylase (HDAC), as valproic acid an HDAC inhibitor did not induce intracellular IL-4 production.

**Conclusions:** Butyrate induces IL-4 production in eosinophils, which is not associated with its HDAC inhibitory activity. Most form of the intracellular IL-4 is non-secreted form of IL-4. Nonetheless, a small amount of IL-4 is secreted. Our results are of potential importance in that eosinophils link gut microbiota to the regulation of immune and metabolic reactions by producing IL-4 in response to butyrate.

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24

### Intraesophageal Administration of Oxazolone to Skin-Sensitized Mice Results in Experimental Eosinophilic Esophagitis

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**Background:** Eosinophilic esophagitis (EoE) is a chronic allergic disease characterized by esophageal dysfunction and infiltration of eosinophils to the esophageal epithelium. Allergic diseases (e.g. atopic dermatitis) and elevated IgE serum levels are considered risk factors for development of EoE. To promote the study of molecular pathways governing the development of EoE and to subsequently advance the development of new therapeutic strategies, an experimental mouse model of EoE is of high interest. Thus, we aimed to establish a robust and reproducible experimental model of EoE.

**Methods:** Mice were sensitized by applying 1% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (OXA) dissolved in acetone on their ears. Seven days after sensitization, mice were ear-challenged with 0.5% OXA in acetone (three times a week for a total of 5 challenges). Twenty-four hours after last ear challenge, serum IgE level was quantified (ELISA). Thereafter, mice were challenged with 1% OXA or vehicle (olive oil: ethanol, 2:1; three times a week for a total of 8 challenges), using plastic feeding tube that has been modified to enable intra-esophageal (and not intragastric) administration. Twenty-four hours after last intraesophageal challenge, the mice were sacrificed, the

## Poster Abstract Session 2: Thursday, 11 July 2019

esophagus was obtained, fixed (4% formaldehyde) and slides containing four esophageal cross sections, from distal to proximal (segments 1 to 4, respectively) were prepared. Thereafter, the slides were stained with hematoxylin & eosin, chloro acetate esterase, anti-major basic protein and anti-Ki-67. In addition, protein from esophagus was extracted for analysis of cytokines by ELISA.

**Results:** Topical ear treatment with OXA resulted in significantly elevated serum IgE levels and the development of atopic skin disease in the ears. Assessment of esophageal eosinophil levels in mice that were intraesophageally-challenged with OXA, revealed significantly elevated lamina propria and intraepithelial eosinophilia in the distal oesophagus, increased expression of Th2-associated chemokines and elevated basal cell proliferation in comparison with control mice. In addition, intraesophageal-challenged mice displayed markedly thickening of the lamina propria. Lamina propria thickening was correlated with the levels of esophageal eosinophils. No differences were observed in the levels of esophageal mast cells.

**Conclusion:** We have established a reliable experimental model for experimental EoE that could be further used to mechanistically dissect molecular pathways and the pathogenesis of EoE.

26

### Human Neutrophils Release Extracellular DNA Traps in Response to *Histoplasma Capsulatum Var.Capsulatum*

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**Background:** Neutrophils are the first line of defense against invading microorganisms. These cells eliminate both intra and extracellular pathogens by mechanisms such as phagocytosis, degranulation and release of neutrophil extracellular traps (NETs). *Histoplasma capsulatum var.capsulatum* (*HC*) is a thermally dimorphic fungus with a worldwide distribution that causes histoplasmosis, a systemic disease that can affect both immunocompromised and immunocompetent individuals. The release of NETs has been described as an important host defense mechanism against different fungi, however there are no reports demonstrating that this process would be implicated in *HC* infection. So, the aim of this work is to investigate whether isolated human neutrophils release NETs in response to *HC* and the mechanisms involved.

**Methods and Results:** Neutrophils were isolated from blood of healthy donors. Cells were then stimulated with *HC* in different ratios (fungus:cell) 1:1, 5:1, 10:1. The release of NETs was evaluated at different incubation times by a quantitative fluorimetric method and by confocal fluorescence microscopy. We found that *HC* was capable to substantially induce the release of NETs. The incubation time of 6h and the ratio (fungus:cell) 10:1 were selected for further studies. Pretreatments of neutrophils for 30 min with DPI, an inhibitor of reactive oxygen species (ROS); with PP2, an inhibitor of the family Src kinase; piceatannol and OXSI, inhibitors of Syk tyrosine kinase; and the AKT inhibitor VII, inhibitor of AKT, significantly inhibited the *HC*-induced NETs release. Moreover, *HC* induced ROS generation in human neutrophils was abolished by neutrophils pretreatment with PP2 or OXSI (inhibitor of Syk tyrosine kinase).

**Conclusion:** The results indicate that human neutrophils release NETs in response to *HC* through a mechanism dependent of ROS, Src, Syk tyrosine kinase and AKT pathways. In addition, *HC*-induced ROS generation and consequently NETs release seems to be downstream to the activation of Src and Syk tyrosine kinases.

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28

### Regulation of Eosinophil Recruitment in Allergic Airway Inflammation by Tropomyosin Receptor Kinase A

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**Background:** Tropomyosin Receptor Kinase (Trk) A is the high affinity receptor for nerve growth factor. While a role for TrkA signaling has been indicated in allergic airway inflammation (AAI) including asthma, the causal relationship and the underlying mechanisms are unclear. The objective of the current study was to determine the functional role of TrkA signaling in eosinophil recruitment and development of AAI.

**Methods:** TrkA activation and signaling mediated by eotaxin-1 and its role in eosinophil function (adhesion to vascular cell adhesion molecule-1 [VCAM-1], migration) was investigated in bone marrow-derived murine eosinophils using a combined genetic-chemical approach to specifically inhibit TrkA kinase activity with the ATP analog 1-NM-PP1. Airway cellular inflammation, Th1-Th2 cytokine and chemokine

## Poster Abstract Session 2: Thursday, 11 July 2019

level, airway hyperresponsiveness and airway structural changes were evaluated in *Alternaria alternata*-challenged TrkA knock-in (KI) mice treated with 1-NM-PP1.

**Results:** Allergen exposure activated TrkA in airway epithelial cells and recruited inflammatory cells, including eosinophils, in the lungs of mice. Eotaxin-1, a C-C chemokine receptor type 3 (CCR3) ligand, activated TrkA in murine eosinophils as determined by immunofluorescence staining and Western blot analysis. In *in vitro* adhesion and migration assays, blockade of TrkA activation by 1-NM-PP1 enhanced eosinophil spreading on VCAM-1, but markedly inhibited eotaxin-1-induced migration. A pan matrix metalloprotease (MMP) inhibitor inhibited eotaxin-1-induced TrkA activation and eosinophil migration, additively with 1-NM-PP1, suggesting a role for MMPs in TrkA activation. Additionally, blockade of TrkA activation inhibited eotaxin-1-induced calcium flux, ERK(1/2) activation and cell polarization by altering F-actin levels and Cdc42 rearrangement/redistribution. *A. alternata*-challenged TrkA-KI mice treated with 1-NM-PP1 showed marked inhibition of TrkA activation in the lungs along with significantly reduced eosinophilia. In addition, 1-NM-PP1-treated mice exhibited attenuated airway hyperresponsiveness along with significantly reduced levels of IL-5, IL-13 and IL-33 as well as eotaxin-2 in the lungs relative to untreated allergen-challenged mice. Allergen-induced structural changes such as mucus production and airway smooth muscle hyperplasia/hypertrophy were also significantly lower in 1-NM-PP1-treated mice.

**Conclusions:** TrkA is an important player in the signaling pathway activated by eotaxin-1 during eosinophil migration. *In vivo*, TrkA signaling plays an important role in airway recruitment of eosinophils and in promoting structural changes in allergen-exposed airways. Inhibiting TrkA kinase activity can potentially mitigate allergic airway inflammation including asthma by inhibiting eosinophil recruitment and suppressing the development of a Th2 phenotype.

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30

### **Aspergillus Fumigatus-Induced Human Eosinophil Extracellular DNA Traps: Role of IP3K, p38 MAPK and Src Family**

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**Background:** Eosinophils are granulocytes classically involved in allergic diseases and in the host immune responses to parasites. The release of extracellular DNA traps (ETs) by leukocytes is an important mechanism of the innate immune response to pathogens in different infectious conditions, including fungal infections. *Aspergillus fumigatus* (AF) is an opportunistic fungus responsible for the allergic bronchopulmonary aspergillosis (ABPA), a pulmonary disease marked by a prominent eosinophilic inflammation. Previously, we demonstrated that isolated human eosinophils release ETs (EETs) when stimulated by AF *in vitro* in a Syk tyrosine kinase and reactive oxygen species (ROS) independent mechanism. We also characterized the presence of EETs in the mucus plugs of ABPA patients. Now, we aim to elucidate the intracellular signaling pathways that orchestrate the release of EETs in response to AF.

**Methods:** We isolated eosinophils from blood of healthy donors by negative immunomagnetic selection (protocol license number 190/09, HUCFF/UFRJ). Cells were pretreated for 30 minutes with different inhibitors including PP2 (10 $\mu$ M; Src family inhibitor), wortmannin (0,1 $\mu$ M; IP3K inhibitor), AKT inhibitor VIII (2,6 $\mu$ M), SB202190 (10 $\mu$ M; p38 MAP kinase inhibitor), GSK (10 $\mu$ M; PAD4 inhibitor) and AS605240 (10 $\mu$ M; class I IP3K inhibitor), and then stimulated with AF (ratio cell:fungus 1:10) for 6h. The newly purified eosinophils were also stimulated for 6h with different volumes of the supernatant recovered from the culture of eosinophils with AF (ratio 1:10, incubation time 2h). EETs release was evaluated by a quantitative fluorimetric method and confocal microscopy.

**Results:** We observed that the treatment with PP2, wortmannin, SB202190, AKT inhibitor VIII and AS605240 completely abrogated the AF-induced EETs release. These results suggest that Src family, IP3K family (including class I PI3K) and AKT participate in the intracellular mechanism of EETs release. Our results also suggest that p38 MAPK is involved, once the treatment with SB202190 inhibited the AF-induced EETs release. According to the obtained results, no toxicity was observed for the inhibitors used, neither for eosinophils, nor for the fungus. The contact of fresh eosinophils with the supernatant recovered from the incubation of eosinophils with AF did not stimulate the release of EETs, suggesting that the release of these extracellular DNA traps is not dependent on factors secreted by the fungus or the cells. All the results were statistically different.

**Conclusion:** Our results showed that the AF-induced EETs release is dependent on the Src family, IP3K signaling, p38 MAPK and AKT. The mechanism of AF-induced EETs release probably does not involve the recognition of fungus-secreted molecules. Other analyzes are underway to investigate in more detail the mechanisms involved in the release of EETs induced by AF.

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## Improved Diagnosis of Asthma: Metabolomic Profiling of Urine

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**Background:** Obstructive airways diseases like asthma or COPD sometimes show overlapping symptoms that hinder their early and correct diagnosis. Diagnosis of asthma in young children is also more difficult especially in a typical doctor's office. Metabolomics is the study of small molecules created by cellular metabolism. Urine is an ideal biofluid for biomarker discovery being non-invasive and rich in metabolites. Our hypothesis has been that diseases like asthma will create a different metabolomic profile compared to healthy people or those with other diseases like COPD.

**Methods:** <sup>1</sup>H-NMR based metabolomic analysis proposed 53 metabolites as potential diagnostic biomarkers among asthma patients. Our work has developed a novel liquid chromatography-tandem mass spectrometric (LC-MS/MS) platform for targeted analysis of urine biomarkers to better diagnose respiratory diseases like asthma. Metabolites are divided into 3 groups based on chemical structure. The LC-MS/MS methods were validated as per FDA guidelines. Adult subjects (n=150) were recruited from a Respirology clinic and phenotyped based on age, BMI, physician history, medications, smoking status, and lung function data. In regard to Pediatric cohorts, some were recruited from a Pediatric Respirology clinic with similar clinical parameters (n=50). Another cohort was recruited from the CHILD study (n=100). This was a longitudinal birth cohort that measured diagnostic parameters over 5 years. We could not include lung function, but it did have the advantage of respiratory questionnaire data, skin test data, and medications over the 5 year period. It also provided healthy control children. Urine was stored at minus 80 degrees. 40 metabolites were measured in each sample, and the values for each metabolite were referenced to creatinine. The values were analyzed using partial least square-discriminant analysis (PLS-DA, SIMCA) in the context of their working diagnosis.

**Results:** We have developed PLS-DA models for the differentiation of adults with asthma or COPD (R<sup>2</sup>=0.85, Q<sup>2</sup>=0.75), children with uncontrolled asthma (R<sup>2</sup>=0.73, Q<sup>2</sup>=0.59, and preschool children with asthma vs healthy control (R<sup>2</sup>=0.74, Q<sup>2</sup>=0.68). Using blinded test sets, we are reporting encouraging accuracy (>80%) for differentiation of these disease phenotypes.

**Conclusions:** Metabolomic analysis of urine appears to see differences in the clinical presentation of asthma. These data are being validated in future larger patient cohorts as a possible diagnostic test.

**Grant Support:** SHRF, JPCHF, AllerGen, CIHR

## AKST4290, a CCR3 Inhibitor, is Efficacious in Models of Age-Related Eosinophilic Disorders

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**Background:** Eotaxin (CCL11) is a chemokine whose levels in plasma increase with age and that functions as a potent eosinophil chemoattractant. CCR3, the primary receptor for Eotaxin, is primarily expressed on eosinophils, making the eotaxin/CCR3 axis a potential therapeutic target for age-related eosinophilic disorders. Using the CCR3 selective antagonist AKST4290 we aimed to assess effects of modulating eotaxin/CCR3 signaling in both normal mouse aging and an induced model of eosinophilia.

**Methods:** To investigate effects on aging-induced eosinophilia, 6-week old and 18-month old C57BL/6 mice received daily oral treatments of AKST4290 or vehicle for 3 weeks. Whole blood, spleen, and bone marrow were collected for immunological assessments. 8-week old male Hairless (SKH1-Elite) mice were challenged daily for 19 days with the skin sensitizer Oxazolone or vehicle to trigger eosinophilia and inflammation. Challenged mice received daily oral treatments of AKST4290, Dexamethasone or vehicle starting 7 days after the challenge initiation and until study end. Mice were visually scored daily for disease progression and their blood was collected at study end for immunological assessments.

**Results:** AKST4290 treatment reverted blood levels of immune cells affected by aging to levels comparable to those of young mice. Moreover, AKST4290 modulated additional subpopulations of immune cells implicated in age-related dysfunction. In the Oxazolone induced model, treatment with either AKST4290 or Dexamethasone reversed the Oxazolone induced elevation in blood eosinophil levels. These changes were associated with detrimental effects on overall white blood cell counts and critical subpopulations with dexamethasone, but to normal levels with AKST4290. Decreased skin inflammation resulted from treatment with AKST4290 and dexamethasone, alone and in combination.

## Poster Abstract Session 2: Thursday, 11 July 2019

**Conclusions:** AKST4290 is efficacious in old mice at reversing age-associated immune cell level increases, particularly those associated with inflammation. Moreover, in young mice AKST4290 may only target elevated eosinophil levels without further immunologic disruption, as opposed to dexamethasone which evokes a nonspecific and indiscriminate effect that could potentially predispose patients to further adverse effects. In older mice, AKST4290 treatment also reversed multiple age-associated immune changes, while not affecting those in young mice, further supporting AKST4290 normalization of immune modulations induced by aging. These findings implicate AKST4290 as a potent immunomodulator that normalizes immune cell populations to basal levels, and may be efficacious in treating eosinophilic diseases.

36

### The TNF Superfamily Member 14 (TNFSF14/LIGHT) Induces Pro-Inflammatory Responses in Fibroblasts in Eosinophilic Esophagitis

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**Background:** Eosinophilic esophagitis (EoE) is an eosinophil predominant, chronic allergic disease of the esophagus with increasing prevalence. Tissue remodeling is a hallmark complication in EoE and associates with clinical dysphagia and strictures. The Tumor Necrosis Factor Superfamily member 14 (TNFSF14/LIGHT) can drive inflammation and tissue remodeling in asthma, atopic dermatitis, and pulmonary fibrosis. We hypothesized that LIGHT may play a role in EoE remodeling.

**Methods:** We isolated primary esophageal fibroblasts from healthy donors and pediatric patients with active EoE. Cells from 6 different healthy donors and 8 active EoE patients were used in the study. We assessed the expression of the LIGHT receptors Herpes virus entry mediator (HVEM) and lymphotoxin beta receptor (LT $\beta$ R) using flow cytometry. We analyzed the response of healthy and diseased fibroblasts to LIGHT versus vehicle or TGF- $\beta$ 1 and to LIGHT in combination with TGF- $\beta$ 1.

**Results:** HVEM and LT $\beta$ R were expressed on healthy donors and active EoE patient fibroblasts. LIGHT had multifactorial effects on human fibroblasts and significantly induced the expression of chemokines CCL5 (10.49 fold,  $p=0.0001$ ) and IL-6 (3.05 fold,  $p=0.016$ ), intracellular adhesion molecule 1 (ICAM1) (5.313 fold,  $p=0.0011$  in healthy FBLs; 7.15 fold,  $p<0.0001$  in active EoE FBLs), and IL-33 (6.23 fold,  $p=0.0001$  in healthy FBLs; 8.79 fold,  $p=0.037$  in active EoE FBLs). Of note, while healthy fibroblasts had nuclear IL-33 protein that was increased with LIGHT, EoE fibroblasts had essentially undetectable nuclear IL-33 despite LIGHT treatment. Treatment with TGF $\beta$ 1 increased HVEM expression in healthy and active EoE FBLs. Subsequent treatment with LIGHT further augmented ICAM1, suggesting that LIGHT effects in esophageal fibroblasts may occur through HVEM. LIGHT induced phosphorylation of p38 and nuclear translocation of p65 NF- $\kappa$ B subunit, invoking the NF- $\kappa$ B and MAP kinase pathways.

**Conclusions:** Our studies support a role for the TNF superfamily member, LIGHT, as a potential upstream regulator of EoE pathogenesis. These data suggest a mechanistic network between TGF- $\beta$ 1 and LIGHT that drives EoE-relevant activities in fibroblast including promoting adhesion molecules, chemokines and inflammatory cytokines, and alarmins such as IL-33. As such, LIGHT may serve as a novel therapeutic target in chronic inflammatory EoE.

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38

### Lysophosphatidylserine Induces Eosinophil Extracellular Trap Cell Death

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**Background:** Eosinophil extracellular trap (EET) is extension of DNA fiber containing histones and granule proteins to outside of eosinophils and has roles in anti-microbe immune response and pathology of allergic diseases. EET is often accompanied by cell death, dubbed as EETosis. P2Y10, along with GPR34 and GPR174, is a G protein-coupled receptor that is activated by an endogenous lipid mediator lysophosphatidylserine (LysoPS). We have previously demonstrated that human peripheral blood (PB) eosinophils solely express P2Y10 among the three LysoPS receptors and degranulate ECP upon exposure to LysoPS. Importantly, our preliminary study showed that LysoPS triggers eosinophil cell death, as analyzed by LDH release. Accordingly, this study aimed to examine whether and how LysoPS induces EETosis in eosinophils.

**Methods:** PB eosinophils were isolated using eosinophil isolation kit and the purity was more than 95%. Cells were seeded in 8-well chamber slide coated with poly-L-lysine and stimulated by LysoPS in the absence or presence of signaling pathway inhibitors for indicated

## Poster Abstract Session 2: Thursday, 11 July 2019

time intervals. Cells were fixed with 3% paraformaldehyde (PFA), stained with Sytox green and with anti-histone H3/H2A or anti-EPX antibodies, and visualized for EET formation using a ZEISS LSM 800 confocal microscope. EET-forming cells whose nuclei undergo dynamic change evaluated by assigning 4 stages, bilobed nucleus, single nucleus, chromatolysis, and DNA trap, as previously described[1], and expressed as relative fractions (%). Cells were stimulated in the same manner on a 96-well plate, and the culture supernatants were collected to determine cell death (%) using an LDH assay kit.

**Results:** LysoPS induced EET and cell death in dose and/or time dependent manners. EET contained nuclear DNA fiber network with entrapped histones and granule proteins, EPX and MBP. An NADPH oxidase inhibitor diphenyleneiodonium chloride (DPI) or an antioxidant N-Acetyl-L-cysteine (NAC) did not affect LysoPS-mediated EET, indicating the LysoPS-EET was independent of ROS production, as opposed to calcium ionophore A23187-mediated EET. Treatment with pharmacological inhibitors that block pyroptosis, necroptosis, or inflammasomal pathways showed that LysoPS-mediated EET was completely abrogated by pyroptosis inhibitors, partially by necroptosis inhibitors, and marginally by inflammasome inhibitors.

### Conclusions:

1. LysoPS is a potent inducer of EETosis.
2. DNA traps induced by LysoPS include histones and granules on released nuclear DNA fibers.
3. LysoPS-mediated EET is ROS-independent.
4. LysoPS-EET occurs mainly through pyroptosis.

**Grant support:** This work was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2016R1D1A1A09919569 to IYC) and HJK and MSS were supported by Brain Korea 21 Plus.

**Reference:** 1. Shigeharu Ueki et al. Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans (2013) *Blood* 121(11):2074-2083

40

### The Thiazolidinediones as Potential Therapeutic Agents for Eosinophilic Esophagitis Pathologic Remodeling: A Preclinical Evaluation

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**Background:** Eosinophilic esophagitis (EoE) is a T-helper 2 (Th2) disease associated with pathologic tissue remodeling, leading to end-organ dysfunction. During early stage disease, inflammation and subepithelial fibrosis are coupled; but in late stage disease, there can be uncoupling of these features with continued esophageal rigidity and strictures contributing to clinical dysphagia and food impactions. No current interventions directly target esophageal fibrosis. Based on the ability of the thiazolidinediones (TZDs) to regulate intestinal and hepatic fibrosis, we tested the anti-fibrotic effects of the TZDs, rosiglitazone and pioglitazone, in preclinical studies using esophageal fibroblasts.

**Methods:** Fibroblasts were isolated from normal or EoE esophagi and cultured with recombinant TGF- $\beta$ 1 or vehicle in the absence or presence of TZDs. Total RNA was extracted for quantitative PCR. Whole cell lysates were extracted for Western immunoblot analysis. Immunohistochemical analysis of human esophageal biopsies for protein expression of the TZD receptor, peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), was performed.

**Results:** EoE esophageal biopsies and esophageal fibroblasts expressed higher levels of the TZD receptor, PPAR- $\gamma$ , than normal controls. TZD reduced TGF- $\beta$ 1-induced myofibroblast and fibrotic gene and protein expression preferentially in EoE, but not in normal, esophageal fibroblasts. In esophageal fibroblasts, TGF- $\beta$ 1 increased phosphorylated Smad2/3 and p38, but TZDs preferentially inhibited p38 phosphorylation, suggesting signaling pathway-specific effects. The TZDs were more potent than budesonide at decreasing collagen-1a1 and  $\alpha$ -sma expression.

**Conclusions:** The TZDs preferentially exert anti-fibrotic effects in TGF- $\beta$ 1-activated EoE-derived fibroblasts. Our data suggest that TZDs may be of clinical utility in chronic EoE associated with pathologic fibrosis and myofibroblast transformation.

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## Serum Eotaxin-1/CCL11 Levels are Elevated in Severe forms of Systemic Sclerosis

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**Background:** Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease in which strong fibrotic activity leads to end-organ damage. Eosinophils are involved in tissue remodeling/repair and secrete a large number of profibrotic mediators. The presence of eosinophil infiltration and/or specific cationic proteins in damaged tissues (skin, lung, etc.) suggests that these cells have a role in the pathogenesis of SSc and its fibrotic complications. The objective of the present study was to investigate the link between eotaxin-1 (CCL11, the main eosinophil chemokine) and the clinical activity and symptom profile of SSc.

**Methods:** Serum CCL11 levels were compared in a randomly selected cohort of SSc patients vs. a control group of healthy adult subjects. A multivariate linear models statistical approach was used to identify correlations with the SSc patients' biochemical and clinical characteristics. The eosinophil surface expression levels of the CCL11 receptor CCR3 (CD193) were measured by flow cytometry in a second group of 21 SSc patients and a group of 20 healthy subjects. Additional markers surface expression levels were studied in smaller groups (21 SSc patients and 9 healthy subjects), being IL-5R $\alpha$  (CD125), CRTH2 (CD294), HLA Class II, CD11c, CD29, CD32, CD44, CD63, CD69, CD123, CD137 and CD213a1.

**Results:** One hundred and twenty-two SSc patients were prospectively enrolled and compared with 50 healthy controls. After adjustment for age and gender, CCL11 levels were found to be significantly higher in SSc patients than in controls (adjusted mean [95% confidence interval]: 182.7 [166.7-198.7] vs. 144.8 [125.8-163.8], respectively;  $p < 0.001$ ). In multivariate analyses, higher levels of serum CCL11 were associated with a more recent disease onset ( $p = 0.026$ ), more severe symptoms (requiring corticosteroids or immunosuppressants;  $p = 0.036$ ), digital ulcers ( $p = 0.008$ ), a grade 3 or 4 dyspnea ( $p = 0.028$ ), and less articular symptomatology ( $p = 0.012$ ). Furthermore, higher CCL11 levels were associated with older age ( $p = 0.003$ ), and a lower proportion of circulating Eos ( $p = 0.040$ ). These associations remained significant in several sensitivity analyses.

Eosinophil surface expression of CCR3 was significantly lower in SSc patients than in controls ( $p = 0.001$ ) - as observed in some other eosinophilic disorders - and was suggestive of receptor internalization and activation. Similarly, CRTH2, CD63 and CD137 eosinophil surface expression was significantly decreased in SSc patients, with the CD63 and CD137 being even lower in diffuse forms of the disease.

**Conclusions:** Elevated levels of eotaxin-1 are associated with recent-onset, severe and active SSc profiles. Eotaxin-1 elevation in recent disease onsets might suggest the involvement of Eos in the early development of fibrotic lesions and a link with severe vasculopathy. Further studies of eosinophilic involvement in the pathogenesis and natural history of SSc are warranted.

## A Functional Role for Eosinophils in Host Resistance Against *Mycobacterium Tuberculosis*

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**Background:** *Mycobacterium tuberculosis* (*Mtb*) is the leading cause of mortality worldwide due to a single infectious agent. *Mtb* resides in pulmonary macrophages, neutrophils and other phagocytes, which limit bacterial growth. Although eosinophils can have similar effector functions to neutrophils, their role in *Mtb* infection is largely unknown.



## Poster Abstract Session 2: Thursday, 11 July 2019

**Methods:** Eosinophils were purified from normal donors under an IRB-approved protocol (NCT00090662) and stimulated with *Mtb*. Eosinophil responses were assessed by flow cytometry and Luminex assay. Eosinophil responses were quantified in resected lung tissue of TB patients undergoing clinically indicated lung resection surgery, bronchoalveolar lavage from *Mtb* infected rhesus macaques and lung tissue in *Mtb*-infected mice by flow cytometry. For lung resection surgery, the project was approved by the Shanghai Public Health Clinical Center Ethics Committee (2015-S046-02) and all patients provided written informed consent.

**Results:** Eosinophils from healthy human donors exposed to *Mtb* released inflammatory cytokines, degranulated and expressed surface proteins that are associated with activation and migration. Moreover, eosinophils were abundantly present in lung tissue samples from patients with tuberculosis undergoing resection surgery. Eosinophils were significantly increased in the bronchoalveolar lavage fluid of *Mtb*-infected rhesus macaques compared to pre-infection samples, providing evidence that eosinophils are recruited to the lungs in direct response to *Mtb* infection. In fact, within 10 days post-infection, eosinophils were recruited to the lung parenchyma of *Mtb* infected mice in an oxysterol-dependent manner. Most importantly, eosinophil-deficient  $\Delta$ dblGata and PHIL mouse strains displayed increased pulmonary bacterial loads and significantly decreased survival after *Mtb* infection.

**Conclusion:** Taken together, these data argue for a previously unrecognized protective role of eosinophils in host resistance against *Mtb* infection. We are currently exploring the underlying mechanisms of eosinophil-mediated host resistance against *Mtb*.

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46

### Identification of Reactive Oxygen Species Production Site in Siglec-8 Induced Eosinophil Death

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**Rationale:** Siglec-8 is expressed on the surface of eosinophils and induces cell death. This function is paradoxically enhanced by co-stimulation with IL-5, an activation/survival factor for eosinophils. We previously reported that this cell death was dependent on intracellular reactive oxygen species (ROS), contrary to eosinophil ETosis which was mostly extracellular ROS dependent. Since NADPH oxidase (NOX) is a major source of ROS in eosinophil and dysfunction of NOX has been implicated in several types of inflammatory bowel diseases in which eosinophil accumulation is frequently observed, we hypothesized that defective ROS production from NOX might lead to insufficient Siglec-8-mediated cell death and disease progression in such diseases. To evaluate this hypothesis, we conducted functional analysis of NADPH oxidase (NOX) and mitochondria to identify the source of intracellular ROS production in Siglec-8 induced cell death.

**Methods:** Human peripheral blood eosinophils were purified and stimulated by anti-Siglec-8 monoclonal antibodies (as ligand) simultaneously with IL-5, or by  $Ca^{++}$  ionophore A23187 as ETosis inducer. Then we evaluated (1) phosphorylation of p40 *phox* / p47 *phox* (NOX cytosolic subunits) by Western Blotting and phospho-flow, (2) cell surface expression of gp91 *phox* (a NOX membrane subunit) by flow cytometry, (3) mitochondrial membrane depolarization and its ROS production using fluorescent indicators JC-1 and MitoSOX Red, respectively.

**Results:** Phosphorylation of p40 *phox* was observed with both Siglec-8 and A23187 stimulation, with the latter showed more prominent change. p47 *phox* activation were found to be similar for both stimulations. Interestingly, cell surface expression of gp91 *phox* was not changed by Siglec-8, contrary to the significant increase by A23187. In mitochondrial analysis, mild depolarization but no ROS production was detected in Siglec-8 stimulated cells.

**Conclusions:** NOX was turned out to be the predominant source of intracellular ROS in Siglec-8 mediated cell death. The difference in surface expression of gp91 *phox* may explain the difference in ROS production by Siglec-8 and ETosis stimuli, proposing previously unrecognized property of eosinophil NOX

48

### Efficacy and Tolerance of Interferon- $\alpha$ in Hypereosinophilic Syndromes. A Retrospective, Multicenter Study on 29 Patients

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## Poster Abstract Session 2: Thursday, 11 July 2019

**Background:** Corticosteroids and imatinib are the first line treatment in hypereosinophilic syndromes (HES) according FIP1L1-PDGFR $\alpha$  (F/P) positivity. In HES unrelated to F/P, second line available treatment are historically interferon alpha (IFN- $\alpha$ ) and hydroxyurea, while anti-interleukin-5 antibodies are currently investigated. Data concerning efficacy of IFN- $\alpha$  in HES are limited to small case series and one large international study with a short-term follow-up.

**Methods:** We performed a multicenter, retrospective, descriptive study to evaluate the efficacy and safety of IFN- $\alpha$  in F/P-negative HES patients. Complete hematologic responses (CHR: eosinophils < 0,5 G/L) and partial hematologic responses (PHR: eosinophils between 0,5 to 1,5 G/L or a decrease of more than 50%), as well as tolerance and corticosteroid sparing-effect were analyzed at 1, 3, 6 and 12 months.

**Results:** Twenty-nine patients (9 women, with a mean [+/-SD] age at diagnosis of 38 [+/-13] years were included. Fifteen patients (52%) had idiopathic HES, 10 (34%) had a lymphocytic HES, and 4 (14%) had a clonal HES. The most common clinical manifestations were cutaneous (69%) and gastrointestinal involvement (31%). IFN- $\alpha$  was used in pegylated form in 16 patients at an average dose of 113 [+/-45  $\mu$ g] /week and in non-pegylated form in 13 patients at an average dose of 12 [+/-9] MIU / week. At one month, a CHR or PHR was observed in 24 patients (83%), including 16 in CHR. At 1 year, 16 patients were still treated with IFN- $\alpha$ , (9 in CHR and 7 in PHR). Treatment with IFN- $\alpha$  was associated with a significant decrease in the mean daily prednisone dose from 20 [+/-16] mg/day at inclusion to 11 [+/-6] mg/day at 6 months and at 9 [+/-9] mg/j at 1 year. After a median of 15 months (range 1 to 161 months), 21 patients (72%) discontinued IFN- $\alpha$  mainly due to adverse events (27%) or loss of efficacy (31%). At the end of the follow-up, 8 patients were still on IFN- $\alpha$  with a median duration of treatment of 21.5 months (3 to 161 months), including 6 in CHR and 2 in RHP. In these 8 patients, pegylated IFN- $\alpha$  was used at the median maintenance dose of 50  $\mu$ g/week with maintenance of a corticosteroid sparing-effect (median daily prednisone dose at 5.5 mg/day).

**Conclusions:** IFN- $\alpha$  appears to be an effective treatment for corticosteroid-resistant or corticosteroid-dependent F/P-negative HES. Its effectiveness can be maintained over long term and at low dose in nearly a quarter of patients, with good tolerance. However, a loss of efficacy or adverse effects justify discontinuation of IFN- $\alpha$  in the majority of cases, underlying the need of alternative therapies.

50

### Eosinophil Subsets in Pediatric Exacerbation-Prone Asthma

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**Background:** Eosinophilic asthma has been identified as a distinct asthma phenotype that is associated with severe asthma and increased risk of asthma exacerbations. Treating patients with severe eosinophilic asthma with biologic therapies that specifically target eosinophils and/or inhibit eosinophil-promoting pathways has been very effective in reducing exacerbations and improving lung function in adolescent and adult patients. Identifying unique surface characteristics of eosinophils from patients with severe eosinophilic asthma will likely help understand the pathogenesis of this disease and assist providers in selecting personalized treatment approaches.

**Methods:** Peripheral blood eosinophils and neutrophils from 81 children with moderate-to-severe asthma enrolled in the Mepolizumab Adjunctive Therapy for the Prevention of Asthma Exacerbations in Urban Children (MUPPITS-2) were evaluated at baseline for surface expression of Siglec-8, CD9, CD11a, CD44, CD45RO, CD48, CD69, CD89, CD125, and CCR3 by flow cytometry. Each surface marker was stained individually, and the median fluorescent intensity was normalized to control staining for that sample (n-MFI). A subset of samples was also stained for IgA on the cell surface. Peripheral blood samples from a second independent cohort of pediatric patients seen in the clinics at our institution with mild or severe eosinophilic asthma were evaluated for surface expression of CD89.

**Results:** A subset (27/81, 33%) of individuals had 2 populations of eosinophils expressing CD45RO with one subset with almost 2-fold higher CD45RO n-MFI (14303 vs 7320,  $P < 0.0001$ , Mann Whitney). The eosinophils from the individuals with 2 populations of CD45RO<sup>+</sup> eosinophils, also as a group had higher CD125/IL-5R $\alpha$  surface expression compared to eosinophils from individuals with a single CD45RO<sup>+</sup> population (1244 vs 962 n-MFI,  $P = 0.005$ , Mann Whitney). In addition, the eosinophils from individuals in the MUPPITS-2 cohort with the highest CD125 expression also had higher CCR3 (1068 vs 922 n-MFI,  $P = 0.0075$ ), CD9 (18669 vs 12335 n-MFI,  $P = 0.0015$ ) and CD89 (773 vs 536 n-MFI,  $P = 0.019$ , Mann Whitney) surface expression. In addition, we noted 3 unique subsets of individuals in the MUPPITS-2 cohort with regard to surface expression of CD89, the IgA receptor, including a subset (61/81) of individuals with CD89-expressing neutrophils and eosinophils, a second subset (13/81) with CD89-expressing neutrophils, but no expression by their eosinophils, and a third

## Poster Abstract Session 2: Thursday, 11 July 2019

subset (7/81) with no CD89 expression by their eosinophils or neutrophils. We reproduced the same 3 unique patterns of CD89 surface expression in our independent cohort of children with mild or severe asthma. CD89 expression was associated with IgA detected on the cell surface.

**Conclusion:** The surface phenotype of eosinophils from children with exacerbation-prone asthma revealed eosinophil subsets within individuals (high CD125) and identified subgroups of individuals based on differential expression of CD45RO and CD89 by their eosinophils. Future work will determine whether surface expression patterns are associated with specific clinical characteristics (e.g. lung function, total IgE), IgA serum levels, and response to therapy with mepolizumab.

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52

### Eosinophil Associated Type-2 Gene Expression in Childhood Asthma

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**Background:** Excessive Th2-type (T2) inflammation has been shown to play an important role in the immunopathogenesis of asthma, and beneficial effects in exacerbation prevention have been seen with monoclonal antibodies that target T2 and eosinophilic inflammation. However questions remain as to the mechanisms by which T2 inflammation promotes disease, and why these targeted therapies prevent only a subset of exacerbations.

**Methods:** 165 children (6 to 17 years old) with difficult-to-control, exacerbation-prone asthma (requiring at least 250mcg bid inhaled fluticasone, or equivalency, and history of 2 exacerbations requiring systemic corticosteroids in the past 12 months) and blood eosinophils  $\geq 150/\text{mm}^3$  were enrolled. Upper airway lavage and blood samples were collected at baseline during a period of asthma control and children were monitored for upper respiratory infection (URI) symptoms and asthma exacerbations. Repeat samples were collected during illnesses. Airway and blood gene expression was assessed by RNA-sequencing and viral infections by PCR. Differential gene expression was assessed by serial cell deconvolution, modular analysis, and linear modeling.

**Results:** A module of genes expressed in the airway samples was significantly linked with airway eosinophil numbers through cell deconvolution ( $p < 0.001$ ) and included the T2 cytokines IL-4, IL-5, and IL-13, as well as transcription factors GATA1 and GATA2. Logistic regression demonstrated that the level of expression of this module in the airway at the baseline healthy visit was significantly associated with the risk of developing an exacerbation in the subsequent 30 days (OR 2.17;  $p < 0.001$ ) but not with the overall frequency of exacerbations. This module also had a significant inverse correlation with the expression of airway type 1 interferon (T1-IFN) response genes ( $p < 0.001$ ) at the baseline visit. Similarly blood eosinophil numbers, though not blood gene expression, were significantly associated with the risk of developing an exacerbation in the subsequent 30 days (OR 1.172;  $p = 0.007$ ). Furthermore the blood eosinophil percentage during a reported URI was significantly associated with the decrease in FEV<sub>1</sub> percent predicted ( $\Delta\text{FEV}_1\%$ ) from baseline during the illness ( $p = 0.004$ ).

**Conclusions:** In a cohort of children with exacerbation-prone asthma, elevated airway expression of a network of eosinophil-associated T2 genes and elevated blood eosinophils predict short-term exacerbation risk, but not the overall frequency of exacerbations. This data suggests a specific "at-risk" immune state, during which a child is most likely to experience an exacerbation. Furthermore, airway T2 inflammation is inversely associated with T1-IFN gene expression, suggesting this airway T2 inflammation may impair the antiviral response as a potential mechanism of increased exacerbation risk.

## Improvement in Quality of Life (QOL) in Pediatric Patients with Eosinophilic Esophagitis and Food Allergy after Initial Clinic Evaluation

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**Background:** The aim of this study was to determine change in quality of life for pediatric patients with eosinophilic esophagitis (EoE) or IgE mediated food allergy after their initial clinic visit, which included education about rescue medications and food allergy action plans.

**Methods:** The Pediatric QOL Inventory (PedsQL™) survey was given to patients ages 2–18 years (or associated caregiver) referred for food allergies or EoE in the Texas Children's Hospital Allergy/Immunology Clinic or Eosinophilic Gastrointestinal Clinic. Surveys were completed at the initial visit and again at follow up visit at least 3 months later. 344 patients were recruited between 2014-2017. Measured outcomes of the survey included comparison of QOL score among various demographic characteristics and comparison of initial to follow up visit scores in physical, social, emotional & school functioning domains. Higher scores correlated with better QOL.

**Results:** Initial surveys were completed by 344 subjects (patient or caregiver), while follow-up surveys were completed by 104 subjects. On initial visit, EoE patients scored significantly ( $p < 0.05$ ) lower than food allergy patients in both total score and physical functioning score. Medicare patients also scored significantly lower than private insurance patients. When comparing QOL at follow-up versus initial visit, there was a subset of patients who had improved QOL ( $p = 0.087$ ). Race was shown to be a significant factor in the change of physical and school functioning scores at the follow up visit.

**Conclusions:** When evaluating quality of life of pediatric patients with food allergy associated disease, there is a significant difference in QOL between patients with EoE versus food allergy, particularly in the domain of physical functioning. A trend was observed showing improvement of QOL in a subset of children after allergy/immunology specialist intervention.

## IL-33 Expression and Potential Activation of Eosinophils Is Associated With ORMDL3

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**Background:** We, along with others, have previously shown that IL-33, a pro-inflammatory cytokine, stimulates eosinophil through their membrane-bound receptor ST2. This stimulation promotes eosinophil survival and increases adhesion and degranulation. We have also previously shown that patients with the at-risk genotype at the 17q21 locus (near ORMDL3, Orosomucoid like 3, gene) have greater numbers of blood eosinophils. We sought to determine whether there is a relationship between ORMDL3 expression and IL-33 expression in epithelial cells, which would subsequently affect eosinophil activation.

**Methods:** In an IRB approved protocol, fresh blood eosinophils and primary nasal epithelial cells were collected from human subjects 18-55 years, most with an allergic rhinitis diagnosis with or without mild asthma, and who were genotyped at the 17q21 locus (rs7216389). Eosinophils were stimulated with either IL-33 (1ng/mL), IL-5 (10ng/mL), IL-3 (10ng/mL), or eotaxin-1 (10ng/mL) for 1 to 4 hours. Eosinophil surface markers were analyzed by multicolor flow cytometry. Adhesion of eosinophils was assessed by culture on wells coated with VCAM-1, periostin, and ICAM-1. Primary nasal epithelial cells were passaged 2-3 times and grown to confluence before ORMDL3 was knocked down using siRNA (5nM) for 24 and 48 hours. ORMDL3, IL-33, and ST2 RNA were analyzed through qPCR (Taqman). Western blotting was utilized for protein detection of IL-33 (goat  $\alpha$ -IL-33, Clone # 40015C, R&D systems) and ORMDL3 (rabbit  $\alpha$ -ORMDL3, Lot # 2789878, EMD Millipore).

**Results:** Eosinophil stimulation with IL-33 resulted in increased surface expression of CD11b, CD18, CD66b, and ICAM-1 markers. IL-33 also stimulated adhesion to ICAM-1 and periostin substrates. In primary nasal epithelial cells, IL-33 expression decreased with the siRNA knockdown of ORMDL3 at both 24 and 48 hours. The siRNA knockdown of ORMDL3 resulted in 5-fold decrease in ORMDL3 mRNA and we confirmed a 2-5 fold decrease in protein levels. Notably, the decreased ORMDL3 expression was also associated with a 5-fold decrease in IL-33 mRNA and corresponding a 2-5 fold decrease in IL-33 protein levels.

**Conclusion:** IL-33 is an activator of eosinophils and one of its primary sources is from epithelial cells. In experimental conditions of lowered ORMDL3 mRNA and protein expression in epithelial cells, there is an associated decrease in IL-33 mRNA and protein levels. We speculate that ORMDL3 is involved in the risk for allergic disease due to its effect on expression of IL-33 and subsequent activation of eosinophils.

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## Quantifying Mucosal Eosinophilic Inflammation in EoE USING the 1-Hour Esophageal String Test

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**Background:** Eosinophilic esophagitis (EoE) is a chronic eosinophil-mediated inflammatory disease of the esophagus with an increasing prevalence in both children and adults. To date, invasive endoscopy with biopsy is the only method used to diagnose EoE and follow patient responses to treatment. Food elimination diets have been shown to be highly effective in resolving EoE histopathology and symptoms; however, such treatment strategies in children require multiple sedated endoscopic procedures that are unpleasant, costly, and with possibly deleterious effects. We have developed a minimally invasive method to capture and quantify biomarkers of eosinophilic inflammation using a 1-hr version of the Esophageal String Test (EST).

**Methods:** In a multi-site observational study, a total of 143 pediatric and adult subjects with active EoE, inactive EoE, GERD, or normal esophageal mucosa were assessed for eosinophil-associated proteins (MBP-1, CLC/Gal-10, EPX, EDN, Eotaxin-2, Eotaxin-3) both by EST and biopsy. Protein levels in string and biopsy extracts were quantified using either in-house developed or commercially available ELISAs. Preliminary longitudinal assessment of subjects undergoing treatment using a 4 food (milk, wheat, soy, and egg) elimination diet (FFED) or topical steroid was done on 5 pediatric EoE patients. Subjects were assessed after food elimination for 6-8 weeks and following food reintroductions after 4-6 weeks.

**Results:** The EST successfully captured eosinophil-associated proteins from the mucosa in a 1-hour dwell time within the esophagus. Protein levels detected by the EST were significantly correlated ( $p < 0.0001$ ) with both histologic peak eosinophil counts (Eos/HPF) and the levels of the same biomarkers quantified in biopsy extracts. The correlation between protein levels detected by EST and peak eosinophil counts was highest for Eotaxin-3 ( $r=0.61$ ), followed by EPX ( $r=0.51$ ), EDN ( $r=0.46$ ), CLC/Gal-10 ( $r=0.40$ ), MBP-1 ( $r=0.40$ ), and Eotaxin-2 ( $r=0.40$ ). Protein levels detected by EST or quantified in biopsy extracts had the highest correlation for eotaxin-3 ( $r=0.73$ ), followed by eotaxin-2 ( $r=0.60$ ), EPX ( $r=0.49$ ), MBP1 ( $r=0.46$ ), EDN ( $r=0.45$ ) and finally CLC/Gal-10 ( $r=0.36$ ). The preliminary longitudinal assessment of pediatric EoE patients undergoing treatment using FFED or topical steroid, showed that the EST can be used to effectively monitor changes in eosinophil-associated protein biomarkers that occur with treatment. The changes in biomarker levels were associated with changes in the numbers of histologic peak eosinophil counts in mucosal biopsies, showing comparable percent changes in biomarker levels in ESTs vs. biopsy extracts, and despite the small sample size, showed a number of statistically significant correlations with histologic eosinophil counts (e.g. MBP-1: Spearman  $r=0.753$ ,  $p=0.031$ ; EPX:  $r=0.805$ ,  $p=0.029$ ).

**Conclusion:** The 1-hour EST is a minimally invasive, effective, and safe method for the detection of eosinophil-associated protein biomarkers of eosinophilic inflammation of the esophagus and could also be used to track changes in mucosal inflammation in EoE patients undergoing FFED treatment, both after food elimination and during food reintroductions.

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## Elevated Serum Tarc in Patients with Idiopathic Hypereosinophilic Syndrome: A Marker of Lymphocyte- Driven Disease?

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**Background:** High serum levels of thymus-and-activation-regulated-chemokine (TARC) have been reported in subjects with CD3-CD4+ T cell-associated lymphocytic variant hypereosinophilic syndrome (L-HES). In vitro studies have shown that IL-4R engagement contributes to TARC production in response to these aberrant T cells, suggesting a direct role of their type-2 cytokine profile.

Elevated serum TARC levels may also be detected in a subset of subjects with idiopathic HES (i-HES), and this biomarker is associated with a suboptimal response to anti-IL-5 therapy, whether aberrant T cells are detected or not. This study explores underlying pathogenic disease mechanisms and potential role of type-2 cytokines in i-HES patients with high serum TARC levels.

**Methods:** Serum mediators, and circulating and tissue lymphocytes were studied in 14 patients with i-HES and serum TARC levels above 1000 pg/ml (hi-TARC), using healthy subjects and i-HES patients with low TARC levels as controls. Serum IL-5 and sCD25 were measured by ELISA. T cell immunophenotyping, quantification of ILC2s, investigation of TCR V-beta repertoire, and quantification of intracytoplasmic cytokines were performed by flow cytometry on circulating leucocytes. The TCR $\gamma$  chain gene rearrangement analysis was performed on whole blood and purified CD8+ and CD8- cells using the LymphoTrack® Dx TRG Assay. PBMC were cultured in presence of PMA+A23187 (48h), PMA+anti-CD28 (48h), and anti-CD3/anti-CD28 (5d), and cytokines were measured in supernatants by ELISA. Presence of cells (CD4, CD8) and mediators (TARC, IL-33) was investigated in skin biopsies by immunohistochemistry.

**Results:** Serum IL-5 was detectable in 4/14 hi-TARC, 1/6 lo-TARC, and 0/12 control subjects. A weak but significant correlation was observed between serum TARC and soluble CD25 levels ( $r$  0.5  $p$  0.008). Immunophenotyping of blood T cells did not reveal differences in distribution among T cell subsets, or increased presence of anomalies previously reported in HES (e.g. CD3+CD4-CD8-) in hi-TARC patients. A significantly increased proportion of activated (HLA-DR+) CD8 T cells was found compared to age-matched healthy controls (43.5 vs 26%,  $p$  0.02), and a trend towards increased terminally differentiated effector CD27-CD28- CD8 cells ( $p$  0.07). Percentages of CD4 T cells expressing CD7, CD25 and CD27 did not differ.

Clonal TCR gene rearrangement patterns were detected in whole blood in 50% of cases. Immuno-magnetic purification established that clonality concerned the CD8 T cell subset all cases except one. Analysis of the TCR V-beta repertoire by flow cytometry showed frequent over-representation of V-beta families mostly within the CD8 T cell subset, but did not show any specific families that were consistently expanded.

In vitro stimulation of PBMC and/or purified CD3 T cells with PMA-ionophore showed detectable IL-5 in culture supernatants from 5/5 untreated hi-TARC patients, but not from healthy controls. No differences in type-2 cytokine levels were observed in supernatants following prolonged stimulation with anti-CD3+anti-CD28. Intracytoplasmic expression of IFN-gamma was significantly lower in CD4 T cells (10.7 vs 27.3%,  $p$  0.004) from hi-TARC subjects, but no differences were observed for type-2 cytokines.

The proportion of ILC2 cells among circulating CD45+ lymphocytes was significantly lower in hi-TARC patients, and an inverse correlation was observed between serum TARC and blood ILC2 counts ( $r$  -0.67,  $p$  0.0004). An inverse correlation between serum TARC and blood CD4 T cell counts was also observed ( $r$  -0.48,  $p$  0.04). Tissue biopsies of hi-TARC subjects did not stain for IL-33, but they did contain dense lymphocytic infiltrates that were predominantly CD4+ in 10/12 samples. So far, preliminary experiments with PCR and RNAscope have failed to detect enhanced presence of IL-5 in skin from hi-TARC patients.

**Conclusions:** Contribution of type-2 T cells to pathogenesis of hi-TARC i-HES is suggested by several observations, including increased serum sCD25, presence of dense lymphocytic infiltrates in tissues, enhanced production of type-2 cytokines in vitro and reduced IFN-gamma. The presence of increased contingents of activated clonal CD8 T cells and reduced ILC2 cells in blood suggest possible chronic antigenic stimulation and danger signals triggered by an as-of-yet unknown event, and indicate that this condition involves complex interplay between different cell-types that can be approached by techniques such as multiplex immunohistochemistry.

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## IL-13 Treated Esophageal Epithelial Cells Induce Eosinophil Survival and Activation

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**Background:** Eosinophil recruitment into epithelial tissue occurs in a number of disease such as eosinophilic esophagitis (EoE). The cross-talk between eosinophils and epithelial cells, particularly in the context of EoE, has not been well elucidated.

**Methods:** Human eosinophils were isolated from peripheral blood of healthy donors using dextran and percoll gradients followed by a magnetic-bead based negative selection; purity was regularly >95% as assessed by cyto-spin and flow cytometry. An immortalized human esophageal epithelial cell line (EPC2) was seeded in keratinocyte serum-free media (KSFM) 48 hours before coculture experiments; where stated, IL-13-stimulation (10 pg/mL) was initiated 24h before coculture. Eosinophils ( $2 \times 10^5$  cells) were added to EPC2 cells ( $5 \times 10^5$ ) in 1 mL of media containing equal parts KSFM and RPMI+10%FBS; media was refreshed every 2 days. As a control, eosinophils were cultured in the absence of EPC2 but in the presence of media alone, media supplemented with IL-5 (10 ng/ml), or media supplemented with EPC2 conditioned media (CM) obtained over a 48-hour period from confluent cells. Eosinophil viability was measured by flow cytometry using Annexin-V staining and exclusion of viability dye. Values are represented as mean  $\pm$  standard deviation.

**Results:** Whereas the majority of human eosinophils underwent apoptosis within 4 days of culture in media alone ( $5 \pm 3.8\%$  viability), IL-5-treatment promoted eosinophil viability at 4 ( $96 \pm 1\%$ ), 7 ( $94 \pm 2\%$ ), and 14 ( $52 \pm 11\%$ ) days of culture. In the presence of confluent EPC2 cells, eosinophils were highly viable at 4 ( $94 \pm 1\%$ ) and 7 ( $96 \pm 1\%$ ) days of culture with decreased viability at 14 days ( $30 \pm 6\%$ ). Despite similar viability at 4 and 7 days of coculture in IL-5- and EPC2-treated eosinophils, CD69 expression (as assessed by FACS) was increased (6.79-fold,  $p < 0.05$ ) in coculture but not in the presence of IL-5. Exposure to EPC2 CM increased eosinophil viability at 4 ( $76 \pm 7\%$ ) and 7 ( $39 \pm 5\%$ ) days of culture. Importantly, EPCs treated with IL-13 augmented eosinophil viability at 14 days ( $68 \pm 2\%$ ) significantly more than untreated EPCs ( $p < 0.01$ ).

**Conclusions:** Esophageal epithelial cells promote prolonged eosinophil survival and activation in a manner augmented by IL-13. These findings have implications for understanding EoE and elucidating the bidirectional crosstalk between esophageal epithelial cells and eosinophils.

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## Effects of Sustained Eosinophil Depletion in Humans

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**Background:** Murine models and limited human data suggest that eosinophils play a role in varied immunoregulatory and metabolic processes, such plasma cell maintenance, vaccine recall responses and glucose homeostasis. The long-term effects of profound and sustained eosinophil depletion in humans are unknown. A recent phase 2 clinical trial of eosinophil depletion using benralizumab (anti-IL5R $\alpha$  antibody) in hypereosinophilic syndromes (HES) offers an opportunity to explore this question.

**Methods:** Nineteen HES subjects completed a multi-phase year-long double-blind placebo-controlled trial of monthly benralizumab treatment (NCT02130882). Clinical data and serum samples were collected at baseline and at multiple time points during the trial. Serum cytokines (IL-4, IL-5, IL-13, IL-10, IL-17A, TNF $\alpha$ , IFN $\gamma$ , IL-1 $\beta$ ) and chemokines (TARC, eotaxin-1, -2, and -3) were measured in duplicate at baseline and at several timepoints post-treatment by multiplex analysis. Bone marrow plasma cells, mature (CD20+CD10-) and precursor (CD19+CD10+) B cells in bone marrow aspirates were enumerated at baseline and after three doses of drug versus placebo (week 12) using flow cytometry. Subjects with no history of tetanus vaccination within the past 5 years received Tdap boosters while on benralizumab. Tetanus titers were measured prior to and at six weeks post-vaccination.

**Results:** Benralizumab depleted eosinophils in 17/19 HES subjects, and this effect was sustained to week 48 in 14/19 subjects. After the first three doses of benralizumab therapy (week 12 or 24), serum levels of IL-5, eotaxin-1 and eotaxin-2 were significantly increased compared to baseline values (median change 17.4 pg/mL, 149.3 pg/mL, 1404 pg/mL, all  $p < 0.0001$ ) and remained elevated through week 48. In contrast, eotaxin-3 was decreased as compared to baseline (median change -48.34 pg/mL,  $p = 0.0067$ ) at week 48. After three months of benralizumab therapy, there was no significant change in plasma cell, mature or precursor B cell percentages in the bone marrow aspirates, or in CD3 and CD19 lymphocyte subsets in the peripheral blood. Subjects who received Tdap boosters ( $n = 13$ ) while on benralizumab

## Poster Abstract Session 2: Thursday, 11 July 2019

therapy demonstrated a significant increase in serum tetanus IgG six weeks later (median pre-treatment 0.99 IU/mL, post-vaccination 2.24 IU/mL,  $p=0.0002$ ). Metabolic parameters including body weight and hemoglobin A1c (to assess glucose tolerance) were not significantly changed after the first three doses of drug. None of the subjects developed a parasitic infection or malignancy during the 48 weeks of the trial.

**Conclusions:** In contrast to data from murine models where eosinophils are absent from the time of conception, eosinophil depletion in humans appears to have little effect on plasma cells, vaccine recall responses and glucose homeostasis. Although sustained increases in serum IL-5, eotaxin-1 and eotaxin-2 (and a decrease in eotaxin-3) were observed in HES patients treated with eosinophil-depletion therapy for 48 weeks, the clinical impact of these observations remains to be determined.

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66

### Expression and Function of Type 1 and Type 2 Eosinophils

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**Background:** Eosinophils are evolutionarily conserved granulocytes typically associated with parasite killing or allergic diseases. Although eosinophils are traditionally characterized as destructive and cytotoxic cells with the main activity being degranulation (releasing toxic proteins), we and others are identifying eosinophils as having more diverse roles such as immune regulation in health and disease. We hypothesize that there are many subtypes of immune polarized tissue infiltrating eosinophils that are disease/tissue specific and can be used as a diagnostic/prognostic indicator of health and disease. In particular, we propose type 1 and type 2 immune microenvironments induce specific gene expression and functions of eosinophils.

**Methods:** Blood-derived mouse eosinophils were purified and stimulated *in vitro* with cytokines IL-33/GM-CSF/IL-4 to mimic a type 2 polarized microenvironment (E2) or IFN $\gamma$ /TNF $\alpha$  to mimic a type 1 polarized microenvironment (E1). RNAseq was completed with confirmation RT-PCR and protein expression. Cell surface markers were assayed by flow cytometry. Secondary granule protein release was assayed by EPX ELISA.

**Results:** RNAseq analysis showed E2 had 853 upregulated and 920 downregulated genes and E1 had 698 upregulated and 304 downregulated genes as compared to E0. E2 eosinophils release Th2 mediators, IL-13 and CCL17 and release EPX whereas E1 eosinophils release Th1 associated mediators CXCL10, CXCL9, and release baseline EPX levels. Flow cytometric analysis also shows subtype specific changes in cell surface expression of CD11b, ST2, Siglec-F, CD69, for example.

**Conclusion:** Similar to the stratification of T cells: Th1, Th2, Th17; innate lymphoid cells: ILC1, ILC2, ILC3; and macrophage: M1, M2, etc., eosinophils have the potential for subtype/phenotype differentiation upon exposure to the cytokine microenvironment. Future studies will likely lead to a greater classification of eosinophil subtypes with specific activities in disease and health.

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68

### Eosinophils Suppress Lung Allograft Rejection

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**Rationale:** The role of eosinophils in organ transplant are poorly defined and often associated with negative outcomes. In particular, the standard view of eosinophils as destructive mediators in combination with their presence in rejecting lungs and bronchoalveolar lavages has led to the view these cells are mediators for lung allograft rejection. The immunology of preventing lung rejection is unique to other organ transplants as it relies on the functions of memory CD8 T cells, increased IFN- $\gamma$ , and specific activation of iNOS pathways. Unlike asthma, which is a type 2 environment often rich with eosinophils in the lung, lung transplant is a type 1 environment also rich with eosinophils. Our studies identify novel activities of this transplant recruited eosinophil subtype that enhances lung allograft acceptance.

## Poster Abstract Session 2: Thursday, 11 July 2019

**Methods:** Fully MHC-mismatched Balb/c (H2Kd) lungs engrafted into recipient C57BL/6 (H2kb) mice were measured for eosinophil recruitment, eosinophil cytokine expression and cell surface expression and lung cytokine expression. To assess the role of eosinophils on alloimmunity, Balb/c lung were engrafted into recipient inducible eosinophil-deficient iPHIL C57BL/6 (H2kb) mice that express the human diphtheria toxin receptor only on eosinophils. These mice were either depleted of eosinophils with diphtheria toxin (DT) prior to engraftment or given saline as a control to block recruitment of recipient eosinophils into the donor graft. Transplants were, also, completed with and without co-stimulatory blockade and with or without PDL1 and measured for CD 8 T cell activation. In vitro assays were completed as well to define mechanistic pathways.

**Results:** Eosinophils are recruited into the donor lungs of allograft recipients, are the main producers of iNOS, express type 1 mediators, and increase expression of MHC I and PDL1. We demonstrate that a deficiency of eosinophils results in increased rejection and increased CD8 T effector cells numbers. Significantly, in the absence of co-stimulatory blockade, eosinophil recruitment to the lung is sufficient to suppress alloimmunity. Deficiency in iNOS in eosinophils or blockade of PDL1 on eosinophils results in impaired CD8 T cell suppression and increased allograft rejection.

**Conclusion:** These data provide a novel explanation for the presence of eosinophils in lung allografts that may be more complicated than that of a destructive rejecting cell. Potentially in the early stages of lung allograft, eosinophils act in a manner similar to granulocytic myeloid derived suppressor cells, attenuating the destructive CD8 T cell responses in lung rejection.

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70

### Early Life Represents a Vulnerable Time Window for IL-33-Induced Lung Pathology

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**Background:** IL-33, an IL-1 family cytokine, is constitutively expressed in mucosal tissues and other organs in healthy humans and animals, and expression levels increase in inflammatory conditions. Although IL-33-mediated promotion of type 2 immune responses has been well established, a gap in our knowledge regarding the functional diversity of this pleiotropic cytokine remains. In this study, we sought to investigate whether ages make any differences in immunologic and pathologic effects of IL-33.

**Methods:** We developed a new IL-33 transgenic mouse model in which overexpression of full-length IL-33 is induced in lung epithelial cells at different developmental stages.

**Results:** In adult mice, an approximately 3-fold increase in the steady-state IL-33 levels produced no pathologic effects in the lungs. When exposed to airborne allergens, adult transgenic mice released more IL-33 extracellularly and exhibited robust type 2 immune responses. In neonatal transgenic mice, up to postnasal day 14, a similar increase in steady-state IL-33 levels resulted in increased mortality, enlarged alveolar spaces resembling bronchopulmonary dysplasia, and altered expression of genes associated with tissue morphogenesis. Processed 25-kDa IL-33 protein was detected in bronchoalveolar lavage fluids without any exogenous stimuli, and pathologic changes were abolished in mice deficient in the IL-33 receptor ST2.

**Conclusions:** Adult lungs are relatively resistant to IL-33 overexpression unless they encounter environmental insults whereas developing lungs are highly susceptible resulting in pathologic outcomes. Dysregulated expression of IL-33 in early life may be detrimental to the wellbeing of host lungs.

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72

### Multiple Roles of PIN1 in the Regulation of TLR7 Signaling in Eosinophils

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**Background:** Eosinophils (EOS) contribute to respiratory viral clearance from the lung but the mechanisms that promote this antiviral host defense remains unclear. Single-stranded, respiratory viral RNA is predominantly sensed by TLR7 which is highly expressed on immune cells including eosinophils. Previously, we have shown that Pin1, a cis-trans peptidyl-prolyl isomerase (PPIase), regulates EOS bone marrow differentiation, cell survival and pulmonary accumulation in the context of TLR7 activation. Pin1 uniquely interacts with and isomerizes a subset of signaling molecules that contain phosphorylated Ser-Pro or Thr-Pro motifs (pS/pT-P). The isomerization-induced structural change alters target protein stability, function and catabolism.

## Poster Abstract Session 2: Thursday, 11 July 2019

**Methods:** Human eosinophils were purified from blood and Pin1 WT or KO mouse eosinophils were differentiated in vitro from bone marrow. Cells were stimulated with TLR7 agonists for 10 min to 18 h prior to determination of cell shape, migration, and changes in gene and protein expression. The specificity of TLR7 agonists were confirmed with TLR7 KO EOS.

**Results:** Treatment of human and mouse EOS with TLR7 agonists (R848, ssRNA, CL075 and CL307) induced the expression of pro-inflammatory and anti-viral cytokines (IL-3, IL-4, IL-10, TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , and IFN- $\alpha/\beta/\gamma$ ), which were not expressed when cells were pre-incubated with Pin1 or TLR7 inhibitors. Consistent with these observations, TLR7 agonists also failed to induce cytokine expression in EOS from TLR7 KO or Pin1 KO mice. In WT cells, TLR7 agonists also induced cell polarization, F-actin polymerization and migration, which was prevented in WT cells treated with Pin1 inhibitors. Immunoprecipitation (IP)/immunoblotting (IB) revealed that Pin1 specifically interacts with IRAK4 and Rho family of GTPases. Pre-incubation of cells with IRAK4 inhibitors or Rho inhibitors suppressed IFN responses and F-actin polymerization, respectively. It remains to be further explored how Pin1 coordinately controls these phenotypes and the relationship (if any) between cell motility and cytokine gene expression.

**Conclusions:** Pin1 plays multiple roles in the regulation of TLR7-mediated signaling, likely contributing to antiviral immunity and cell migration to the lung in the context of respiratory viral infection.

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74

### Steroid Dose Reducing Effect of Benralizumab, A Monoclonal Antibody to IL-5R $\alpha$ , In Eosinophilic Granulomatosis with Polyangiitis

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**Background:** Eosinophilic granulomatosis with polyangiitis (EGPA) is an eosinophilic vasculitis that affects multiple organs. IL-5 has been implicated in EGPA pathogenesis. Blockade of IL-5 with mepolizumab has resulted in increased remission and reduced glucocorticoid use. We hypothesized that benralizumab, a monoclonal antibody targeting the IL-5R $\alpha$ , would demonstrate efficacy and safety in EGPA.

**Methods:** Ten adult subjects with EGPA and a stable dose of > 5 mg of oral prednisone (or equivalent) were enrolled in this open-label pilot study. After a 4-week run-in period, subjects received subcutaneous injections of benralizumab 30 mg monthly x 3 injections followed by every 8 week dosing x 2 injections. Subjects were permitted to reduce corticosteroid dosing according to a predetermined schedule if stable. This 28-week treatment period was followed by 12-week washout and safety monitoring phases. Exacerbation frequency, % corticosteroid reduction, lung function, biomarkers of inflammation (exhaled nitric oxide, blood eosinophils, IgE), and patient reported outcomes were evaluated. We report reduction in corticosteroid dose and frequency of exacerbation in the three study periods: a) run-in period, b) treatment period, c) washout/ safety monitoring period. Responses between two periods (treatment vs. washout and treatment vs. run-in) were compared using the Wilcoxon Signed-Rank (WRS) test, pairing by subject.

**Results:** Seven of 10 subjects reduced corticosteroid dose by at least 25% from baseline to 1 month after the last injection, with significant difference between the two visits ( $p=0.057$ , one-tailed Signed-Rank test). After benralizumab washout, 5 of 10 subjects maintained or increased corticosteroid dose compared with baseline. Annualized exacerbation rate for subjects was reduced with benralizumab treatment compared to baseline (1.86 during treatment vs. 5.20 during run-in, expressed as means between groups; one-tailed WRS test,  $p=0.16$ ) and compared to the washout period (1.86 during treatment vs. 4.33 during washout, expressed as means between groups; one-tailed WRS test,  $p=0.005$ ). Benralizumab was well tolerated.

**Conclusion:** Anti-IL-5R $\alpha$  therapy with benralizumab safely reduced corticosteroid dosing and exacerbations in patients with EGPA.

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## Oral Corticosteroid Dose Modulation in Severe Asthma: Impact on Peripheral Blood Eosinophil Count

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**Background:** Severe asthma patients often require frequent use or maintenance use of oral corticosteroids (OCS). A portion of these patients have disease driven by eosinophilic inflammation. While eosinophils are sensitive to corticosteroids little is known about the relationship between OCS dose modulation and peripheral blood eosinophil (PBE) count.

The objective of this analysis was to assess the impact of OCS dose modulation on PBE count during the OCS optimization phase of the SIRIUS study.

**Methods:** In the steroid-sparing SIRIUS study, OCS-dependent patients (5-35mg/day) with severe asthma, on a background of high dose ICS plus additional controller(s), were instructed to adjust OCS dose weekly to achieve the lowest effective OCS dose prior to randomization to mepolizumab or placebo. Patients were required to have a PBE count of  $\geq 150$  cells/ $\mu$ L at baseline or  $\geq 300$  cells/ $\mu$ L in the past year. Change in ACQ score from screening triggered OCS adjustment during the OCS optimization phase. In this post-hoc analysis changes in OCS dose and eosinophils during the optimization phase are described.

**Results:** During the optimization phase, and as directed by change in ACQ-5 score, 31 patients (23%) increased, 44 patients (33%) maintained, and 60 patients (44%) decreased OCS dose (Table 1). At the end of optimization the overall group mean OCS dose was 12.8 mg/day and the mean reduction was 2.4 mg/day. The geometric mean ratio blood eosinophil count from beginning to end of optimization was 0.58, 0.80, and 1.52 in those who increased, maintained, or decreased OCS dose, respectively. An inverse relationship between baseline eosinophil count and optimized OCS dose was evident as the subgroup (n=33) with the lowest baseline eosinophil count (<150 cells/ $\mu$ L) had the highest mean optimized OCS dose (15.5 mg/day) and the subgroup (n=39) with the highest baseline eosinophil count ( $\geq 500$  cells/ $\mu$ L) had the lowest mean optimized OCS dose (10.5 mg/day).

**Conclusions:** Following OCS dose optimization in a severe asthma population the OCS dose was inversely correlated to the baseline eosinophil count. A portion of the study population had a baseline eosinophil count  $\geq 500$  cells/ $\mu$ L despite the use of a mean OCS dose of 10.5 mg/day, demonstrating relative peripheral blood eosinophil persistence in the presence of systemic steroids.

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## Eosinophils Dampen Hepatic Ischemia Reperfusion Injury through Interleukine-33 Signaling

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**Background** During orthotopic liver transplantation, hepatic ischemia reperfusion (I/R) injury can cause early graft failure and result in chronic liver rejection or recurrence of viral hepatitis. A significant shortage of donor organs had led to the consideration of using "marginal" livers from older, steatotic, and non-heart-beating donors. However, the utility of "marginal" livers is limited because they are highly susceptible to hepatic I/R injury. Given that the treatment modalities to prevent or attenuate hepatic I/R injury are extremely limited, it is imperative to gain new insights into the mechanisms and uncover novel therapeutic targets. Eosinophils are bone marrow-derived granulocytes that are involved in host defense against parasitic infections and pathogenesis of allergic diseases. Recently, it has been reported that eosinophils are associated with a pathological or protective role in various types of liver injury including chemical-induced injuries, partial hepatectomy in mice. However, the precise role of eosinophils in hepatic I/R injury has not been elucidated.



## Poster Abstract Session 2: Thursday, 11 July 2019

**Methods** 8~10 week-old littermates of WT, PHIL, IL-33<sup>-/-</sup>, ST2<sup>-/-</sup>, and  $\Delta$ dblGATA1 mice were operated with I/R surgery and then sacrificed for phenotype analysis. The plasma of blood was used for biochemical analysis in ALT level. Liver tissues of mice were used to histopathological observation, fluorescence-activated sorting (FACS) and real-time PCR analysis. Adaptive transfer of bone marrow-derived eosinophils (BM-Eos) were performed to mice prior to I/R surgery. Immunostaining was subjected to liver specimens of human biopsy and mice tissue.

**Results** Eosinophils rapidly accumulate in the liver following orthotopic liver transplantation in humans and hepatic IR injury in mice. IL-33 is released during liver I/R injury and its receptor, ST2 is predominantly expressed by eosinophils. Moreover, the IL-33/ST2 signaling in eosinophils mediates their hepato-protective effects. Regarding how eosinophils protect against hepatic IR injury, our data revealed a previously unrecognized interplay between eosinophils and neutrophils. When eosinophils are absent or reduced (in PHIL,  $\Delta$ dblGATA1, IL-33<sup>-/-</sup>, ST2<sup>-/-</sup>, anti-Siglec-F-treated mice), the number of neutrophils in the liver is profoundly increased, coinciding with worsened hepatic I/R injury. In the in vitro and in vivo experiments, ST2, IL-33 stimulates eosinophil production of IL-13, which suppresses neutrophils.

**Conclusion** Based on our findings, rapid accumulation of eosinophils prevents hepatic I/R injury, and the interleukin (IL)-33 signaling through its receptor ST2 (suppression of tumorigenicity 2) as novel approaches to not only improve the clinical outcomes of hepatic I/R injury but also expand donor pool for liver transplantation. This research is supported by NIH grant U01-AA021723-06, R21-AA024636-03, and R01-DK109574-04.







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