
10TH BIENNIAL
SYMPOSIUM
19-23 JULY 2017
GOTHENBURG, SWEDEN
ELITE PARK AVENUE HOTEL
International Eosinophil Society
IES
FINAL PROGRAM

IES-0517-729

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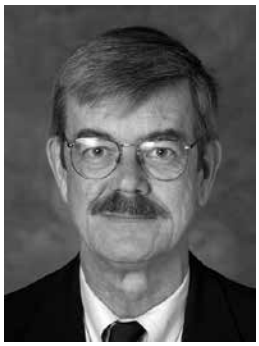
Welcome

Welcome fellow eosinomaniacs! After years of planning, we are excited to host you in Gothenburg for the 10th Biennial Symposium of the International Eosinophil Society, Inc. The 2017 meeting, just like prior ones, should be an ideal opportunity for each of us to explore interests, ideas and collaborations in an environment of openness and collegiality.

For those of you that have attended prior meetings, the structure and format of the meeting should feel familiar, but for those of you attending for the first time, here is a brief overview. Tuesday's pre-conference meeting will be devoted to the 8th in a series of clinically oriented workshops. The workshop organized by Amy Klion, will focus on biologic therapies for eosinophilic disorders. The main meeting will open on Tuesday evening with a reception that will include a talk by Per Venge. Sessions beginning Wednesday morning and continuing until midday Sunday, will highlight the latest advances in basic and clinical research, presented as state-of-the-art talks by leaders in the field, cutting-edge lectures on new findings, and oral presentations and posters taken from over 90 submitted abstracts. There will be plenty of opportunities for discussion during the two poster sessions, and prizes will be given to trainees at the beginning of the last day for best posters and oral presentations.

Finally, we would like to extend our extreme gratitude to our corporate and non-profit sponsors and volunteers, without whom this meeting would never have been possible; to Steve Ackerman, whose efforts once again resulted in our ability to offer travel grants to trainees; for the time and effort of our colleagues among the IES leadership and others who served on the meeting's various committees; and to Kate Filipiak and Kris Heijnen at EDI, whose guidance, professionalism, patience, and skills insured the successful organization of this meeting and made the process such a pleasure.

We hope you enjoy the meeting!



Peter Weller
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Christine Wennerås
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Acknowledgements

IES thanks the following partners for their support of the 10th Biennial Symposium

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General Information

Swedish Coffee Breaks

Coffee breaks are included in the registration fee for attendees and will be served daily. Coffee will be served outside of the Bankett 2 in the Elite Park Avenue Hotel, please check the Scientific Program for exact times.

Education Sessions

All education sessions will take place in the Bankett 2 located in the Elite Park Avenue Hotel.

Meals

Breakfast and Lunch will be provided and included in your registration fee

Poster Sessions

All poster sessions will take place in the Bankett 1. An assortment of beverages and appetizers will be served. Poster presenters will stand next to their posters during their session and be available for questions and discussions.

Registration

The registration desk will be located outside of the Bankett 2.

Hours:

Wednesday, 19 July	7:30-19:30
Thursday, 20 July	7:30-12:30
	15:00-20:00
Friday, 21 July	7:30-13:00
	15:00-20:00
Saturday, 22 July	7:30-13:00
	14:00-17:30
Sunday, 23 July	7:30-12:30

Social Events

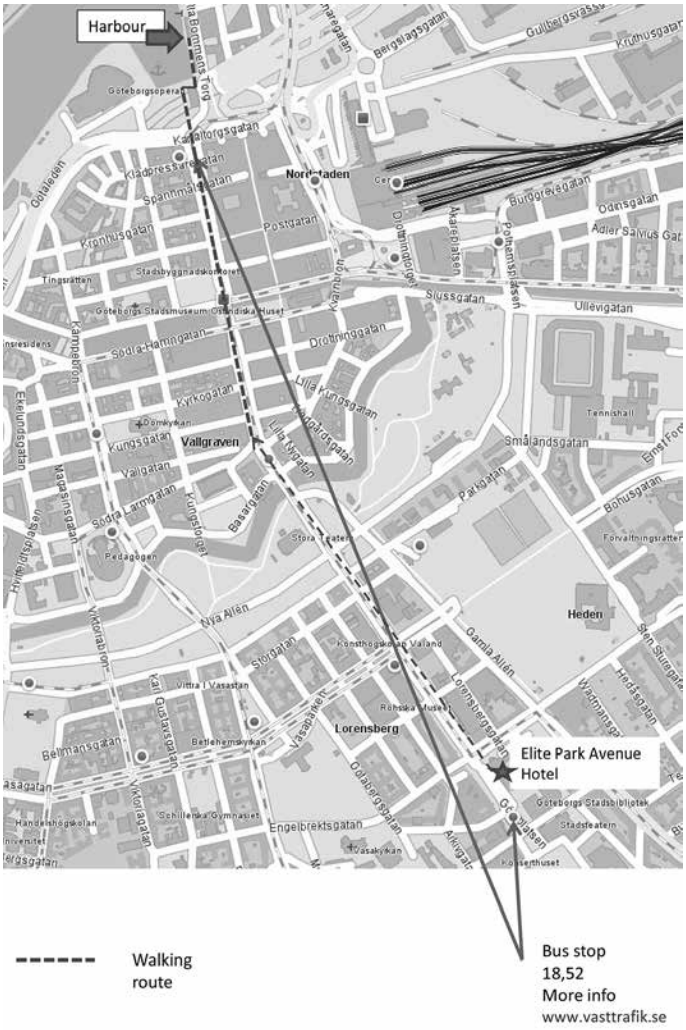
Welcome Reception and Dinner
Wednesday, 19 July, 18:00-19:30

The welcome reception will be held at the Elite Park Avenue Hotel Conference Center. Refreshments and appetizers will be provided.

Symposium Dinner Event and Boat Ride
Saturday, 22 July, 19:00-23:00

The boat ride will depart at 19:00 from the Lilla Bommen dock.

Walking directions from Elite Park Avenue Hotel: Exit hotel and proceed Northwest toward the river along Kungsportsplatsen roughly 20 blocks to dock location. Please see below map for directions or bus location.



Scientific Program

Wednesday, 19 July 2017

RE-TREAD: Progress Towards Meeting the 2012 TREAD Needs in Eosinophilic Research
This workshop was supported in part by funds from the National Institute of Allergy and Infectious Diseases (NIAID).

I. Eosinophil Research: A Status Report

8:30 – 8:40

Introduction and Workshop Agenda – Amy Klion, United States

8:40 – 9:00

Overview of TREAD Objectives – Mike Minnicozzi, United States

II. Where We are Today

Moderator: Hans-Uwe Simon, Switzerland

9:00 - 9:15

Status and Impact of Coding for Eosinophilic Disordersof coding for eosinophilic disorders – Kathleen Sable, United States

9:15 - 10:30

Tissue Markers of Eosinophil Pathogenesis
Histopathology – Margaret Collins, United States
Granule proteins/EETs – Gerald Gleich, United States
Omics – Marc Rothenberg, United States

10:30 - 11:00

Biomarkers in rare eosinophilic diseases
Biomarkers – Joe Arron, United States
Patient-reported outcomes – Paneez Khoury, United States

11:00 - 11:15 Coffee Break

11:15 - 11:45

Mechanisms of Eosinophil Pathogenesis
Animal models – Elizabeth Jacobsen, United States
Granule proteins – Lisa Spencer, United States

11:45 - 12:00

Patient Registries for Rare Eosinophilic Disorders
The French experience – Jean-Emmanuel Kahn, France

12:00 – 13:00 Lunch

13:00 - 13:30

Clinical Trials in Rare Eosinophilic Disorders
What we can learn from rare diseases – Amy Klion, United States
Combining clinical and mechanistic studies – Mike Wechsler, United States

14:00 - 14:30

Therapeutic Agents for Rare Eosinophilic Disorders
Mepolizumab – Jonathan Steinfeld, United States
Dexpramipexole – Calman Prussin, United States

Scientific Program

Wednesday, 19 July 2017

III. Where Do We Go From Here?

14:00 - 14:30

Research opportunites in rare diseases – Rashmi Gopal-Srivastava, United States

14:30 – 16:00

Prioritization of unmet needs and identification of action items
Moderators: Bruce Bochner,United States and Florence Roufosse, Belgium

Welcome and Reception

17:00 - 17:20

Welcome – Peter Weller, United States; Marie Carlson, Sweden; and Christine Wennerås, Sweden

17:20 - 18:00

Eosinophils during two decades: An old friend in new clothes? – Per Venge, Sweden

18:00 - 19:30

Welcome Reception – Elite Park Avenue Hotel Conference Center

Thursday, 20 July 2017

Session 1: Eosinophils in Host Pathogen Defense

Moderators: Helene Rosenberg, United States and Christine Lingblom, Sweden

8:30-9:00

State-of-the-Art: The eosinophils in anti-pathogen host defense – Helene Rosenberg, United States

9:00-9:30

Cutting Edge: Eosinophils and aspergillus fumigatus infection: a DNA trap release story – Josiane Neves, Brazil

9:30-9:45

Abstract Speaker: PIN1 null eosinophils show impaired differentiation and survival after TLR7 signaling – Zhong-Jian Shen, United States

9:45-10:00

Abstract Speaker: Phenotypic and functional changes in eosinophils may promote antiviral responses in hosts with allergic asthma – ali Samarasinghe, United States

10:00-10:30 – Coffee Break

Session 2: Intestinal Eosinophils – What Regulates Them and What Do They Regulate?

Moderators: Steve Ackerman, United States and Maria Lampinen, Sweden

10:30-11:00

State-of-the-Art: Eosinophils regulate homeostasis of the GI tract – Derek McKay, Canada

11:00-11:30

Cutting Edge: The human intestinal microbiome – Ingegerd Adlerberth, Sweden

11:30-11:45

Abstract Speaker: A novel biased antagonist of the eotaxin-CCR3 pathway in eosinophils – Milica Grozdanovic, United States

11:45-12:00

Abstract Speaker: Aiolos regulates eosinophil migration – Patricia Fulkerson, United States

Scientific Program

Thursday, 20 July 2017

12:00-15:00 - Conference lunch, meetings, networking

Session 3: Eosinophils in the Gut - Cause or Protection in Eosinophilic Esophagitis and Inflammatory Bowel Disorders?

Moderators: Marc Rothenberg, United States and Robert Saalman, Sweden

15:00-15:30

State-of-the-Art: Eosinophils in IBD – healers or destroyers? – Marie Carlson, Sweden

15:30-16:00

Cutting Edge: Pathogenesis of eosinophilic esophagitis – Marc Rothenberg, United States

16:00-16:30

Cutting Edge: The eosinophil in eosinophilic esophagitis: the "cell-in-chief" or simply an "assistant" – Alex Straumann, Switzerland

16:30-16:45

Abstract Speaker: A phenotypically distinct subset of eosinophils is recovered with intestinal intraepithelial leukocytes – Evangeline Cornwell, United States

16:45-17:00

Abstract Speaker: Whole exome resequencing identifies putatively functional rare variants associated with eosinophilic gastroenteritis – Andrew Wardlaw, United Kingdom

17:00-17:30 - Coffee Break

Gleich Award

Moderator: Peter Weller, United States

17:30-18:00

Lung-Resident Eosinophils Represent a Distinct Regulatory Eosinophil Subset– Claire Mesnil, Belgium and Stéfanie Raulier, Belgium

Moderators: Francesca Levi-Schaffer, Isreal, Steve Ackerman, United States, Dagmar Simon, Switzerland, Allison Fryer, United States and Patricia Fulkerson, United States

18:00-20:00

Poster Session I (Mini-Oral Presentations, Poster Walkabouts, Drinks and finger foods)

Oral Poster 1 Benralizumab is a Well Tolerated and Effective treatment for PDGFRA-Negative Hypereosinophilic Syndrome – Fei Li Kuang

Oral Poster 2 Eosinophil Peroxidase Increases Thymic Stromal Lymphopoietin Expression in Keratinocytes – Quinn R. Roth-Carter

Oral Poster 3 Lymphocytic Variant Hypereosinophilic Syndrome: Diagnostic Tools Revisited – Caroline A. Carpentier

Poster 1 Composition of Extracellular Matrix Microenvironment Dictates Phenotypic Plasticity, in situ Expansion, Maturation, and Survival of Tissue Eosinophils – Sergejs Berdnikovs

Poster 2 Eosinophils and Morphological Remodeling of the Esophageal Epithelium in Eosinophilic Esophagitis – Simon P. Hogan

Poster 3 Compartmentalization and Vesicular Trafficking of Interferon-Gamma (IFN-?) Within Human Eosinophils – Lívia A. S. Carmo

Poster 4 Rocks Negatively Regulate Secretion of Eosinophil Associated RNAES by Affecting CD11b Integrin – Revital Shamri

Thursday, 20 July 2017

Poster 5 Characterization of a Novel Mouse Strain Expressing Human SIGLEC-8 Only on Eosinophils – Jeremy A. O'Sullivan

Poster 6 Characterizing a Novel Protein in Eosinophils: Nance-Horan Syndrome-Like Protein 2 (NHSL2) – Keren Turton

Poster 7 Regulation Of Eosinophil Granularity By Myb – Jessica E. Bolden

Poster 8 Mouse Eosinophil response to Lipopolysaccharide (LPS) Stimulation – Kennedy Bonjour

Poster 9 Benralizumab in hypereosinophilic syndromes: predictors of response – Fanny A. Legrand

Poster 10 Comparison of Eosinophil Adhesion and Migration Supported by TGF-BETA-Induced Protein (TGFBI) and Periostin Splice Variants – Mats W. Johansson

Poster 11 PI3K/MAPK Blockade Inhibits Eosinophil Extracellular Trap Cell Death – Shigeharu Ueki

Poster 12 For Whom the Eos Toll: Effect of Microenvironment on Murine Eosinophil Toll-Like Receptor Expression – Julia Krumholz

Poster 15 Eosinophil in a Three-Dimensional Collagen Gel in Vitro Have Decreased Surface L-Section (CD62L) – Mats W. Johansson, PhD

Poster 16 Manipulation of Mcl-1 critically alters mouse bone marrow-derived eosinophil apoptosis – Jennifer M. Felton

Poster 17 Adult Eosinophilic Esophagitis Patients' Satisfaction with Different Disease-Specific Treatment Modalities – Ekaterina Safroneeva

Poster 18 Clinical picture and blood eosinophil phenotype of children with collagenous gastritis – Timo Käppi

Poster 19 Does The Grade Of T-Cell Infiltration Correlate With Eosinophil Counts Or Symptoms In Eosinophilic Esophagitis? – Helen Larsson

Poster 20 Initial Clinical Presentation of Patients with Hypereosinophlic Syndrome at a Tertiary Medical Center – Jay Jin

Poster 21 Evaluation of Eosinophil Biomarkers in Eosinophilic Esophagitis Patients – Evelyn L. Angulo

Poster 22 Eosinophilic Gastroenteritis Associated with Hirschsprung's Disease and It's Allied Disorders – Yoshiyuki Yamada

Poster 23 Eosinophilic Esophagitis risk variant at 2p23 dampens IL-13-induced calpain-14 promoter activity in a STAT6-dependent manner – Leah C. Kottyan

Poster 24 Down-regultated eosinophil activity in ulcerative colitis with concomitant primary sclerosing cholangitis – Maria Lampinen

Friday, 21 July 2017

Session 4: Eosinophilic Differentiation and Effector Functions

Moderators: Hans-Uwe Simon, Switzerland and Daniela Janevska Carroll, United States

8:00-8:30

State-of-the-Art: Eosinophil effector functions – Peter Weller, United States

8:30-9:00

State-of-the-Art: Molecular mechanisms regulating eosinophil granule protein release and toxicity – Hans-Uwe Simon, Switzerland

Friday, 21 July 2017

9:00-9:30

Cutting Edge: Checkpoint inhibitory receptors on eosinophils – Ariel Munitz, Israel

9:30-9:45

Abstract Speaker: Trib1 regulates eosinophil identity by restraining the neutrophil identity program – Ethan Mack, United States

9:45-10:00

Abstract Speaker: Exosomes from eosinophils autoregulate and promote eosinophil functions – Victoria Del Pozo, Spain

10:00-10:30 - Coffee Break

Session 5: Eosinophils and Asthma

Moderators: Andrew Wardlaw, United Kingdom and Matthew Drake, United States

10:30-11:00

State-of-the-Art: Latest on eosinophils in asthma – Andrew Wardlaw, United Kingdom

11:00-11:30

Cutting Edge: The role of ILCs in regulating eosinophilic asthma – Roma Sehmi, Canada

11:30-12:00

Cutting Edge: The allergic effector unit: an eosinophil mast cell only pro-inflammatory cross-talk? – Francesca Levi-Schaffer, Israel

12:00-12:15

Abstract Speaker: Vacuolated eosinophils drive maladaptive immunity in experimental allergic asthma through the C5a-C5aR1 axis – Anna Valeska Wiese, Germany

12:15-12:30

Abstract Speaker: When is a wild type mouse not wild type? – Katie M Lebold, United States

12:30-12:45

Abstract Speaker: Mepolizumab for the treatment of patients with eosinophilic granulomatosis with polyangiitis: a phase III randomized, placebo-controlled trial – Michael Wechsler, United States

12:45-15:00 - Conference Lunch, Meetings, Networking

Session 6: Eosinophils in Immune Regulation and Tissue Homeostasis

Moderators: Marie Carlson, Sweden and Lisa Spencer, United States

15:00-15:30

State-of-the-Art: Eosinophils as masters of T cell fate – Christine Wennerås, Sweden

15:30-16:00

State-of-the-Art: Eosinophils as mediators of local immune responses and remodeling – Sergejs Berdnikovs, United States

16:00-16:30

Cutting Edge: Eosinophils suppress Th2 responses in Peyer's patches during nematode infection – Claudia Berek, Germany

16:30-16:45

Abstract Speaker: Single-cell Analysis of Human T cells in Eosinophilic Inflammation – Ting Wen, United States

Friday, 21 July 2017

16:45-17:00

Abstract Speaker: IL-33 dysregulates regulatory T (Treg) cells and impairs established immunological tolerance in the lungs – Hirohito Kita, United States

17:00-17:15 – Coffee Break

Human Vs. Mouse Eosinophils –The Non-Battle of Eosinophils

Moderator: Christine Wennerås, Sweden

17:15-18.00

Pros and cons for elucidating mechanisms of asthma and allergic airways disease – William Busse, United States and Helene Rosenberg, United States

Moderators: Mats Johansson, United States, Josiane Neves, Brazil, Peter Weller, United States, Hirohito Kita, United States and Bruce Bochner, United States

18:00-20:00

Poster Session II (Mini-Oral Presentations, Poster Walkabouts, Drinks and finger foods)

Oral Poster 4 SIGLEC-8 is an Activating receptor on Human Eosinophils for Integrin-Dependent Adhesion, ROS Generation and Apoptosis – Daniela J. Carroll

Oral Poster 5 CD300F Inhibits Adipose Tissue Eosinophil Homing and Subsequent IL-4 Production by Regulating IL-5 Receptor Signaling – Perri Rozenberg

Oral Poster 6 Eosinophils Stimulate Airway Sensory Nerve Growth in Asthma – Matthew G. Drake

Poster 25 Pulmonary Eosinophils Increase Vagus Nerve Mediated Airway Reflex Response in Mice – Zhenying Nie

Poster 26 Eosinophils Regulate Airway Parasympathetic Nerve Ganglion Structure – Alexandra B. Pincus

Poster 27 Ozone and Sensitization Alter Tissue, Lavage and Nerve-Associated Eosinophils in the Lung – Matthew G. Drake

Poster 28 Focal Adhesion Kinase (FAK) Inhibition Blocks Eosinophil Recruitment in response to IL-4 In Vitro and In Vivo – Kamala D. Patel

Poster 29 Dexpramipexole effectively lowers blood and tissue eosinophils in subjects with chronic rhinosinusitis with nasal polyps – Calman Prussin

Poster 30 IL-3 Differentially Activates Eosinophils Compare to IL-5 and GM-CSF – Stephane Esnault

Poster 31 RNA-Sequencing Analysis of Lung Primary Fibroblast response to Eosinophil-Deregulation Products Predicts Downstream Effects on Inflammation, Tissue Remodeling and Lipid Metabolism – Stephane Esnault

Poster 32 Eosinophils Impair Airway Substance P Breakdown by Inhibiting Neutral Endopeptidase – Katie M. Lebold

Poster 33 Quantification of 'Whole Lung' Eosinophilic Inflammation in Asthma and Systemic Disease Using Radiolabelled Autologous Human Eosinophils – Neda Farahi

Poster 35 Increased Beige Fat and Eosinophil Infiltration in Mice Lacking Kruppel-Like Factor 3 (KLF3) – Alexander Knights

Poster 36 Local IL-5 Production by Bone Marrow ILC2s in IL-33-Driven Eosinophllia – Kristina Johansson

Poster 37 Eosinophils Secrete Galectin-10 Via Eosinophilic Extracellular Traps, Exosomes and Immune Synapses – Christine Lingblom

Poster 38 Clinical significance and antigenic specificity of anti-eosinophil autoantibodies – Régis Dieckmann

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Friday, 21 July 2017

Poster 39 Investigation of Natural Killer Cell–Mediated Eosinophil Apoptosis by Benralizumab – Roland Kolbeck

Poster 40 High Affinity IgE Receptor Enhances in vivo Antigen Presentation Capabilities of Airway Eosinophils – Haibin Wang

Poster 41 Potent EOS Adhesion with Il-33 Stimulation and Periostin as Substrate – Paul Fichtinger

Poster 42 Eosinophil Accumlation Oxazolone-Induced Atopic Dermatitis is Independent of IL-13 Receptor Alpha 1 – Almog Bitton

Poster 43 Eosinophil Persistence in the Airway Following Alternaria Inhalation Exposure – Caroline M. Percopo

Poster 44 Eosinophils and Altered Skin Barrier Function in Development of Food Allergy: Novel Mechanisms of Food Allergy – Joan M. Cook-Mills

Poster 45 Short chain fatty (SCFA) acids activate the intrinsic apoptosis pathway in eosinophils – Anna Theiler

Poster 46 I Will Survive: Lung Eosinophils Isolated From Allergen-Challenged Mice Display Prolonged Viability in the Absence of IL-5 – Wendy Geslewitz

Poster 47 Altered miR-155 Expression in Allergic Asthmatic Airways – Madeleine K. Radinger

Poster 48 Evaluation of immunohistopathologic and therapeutic effects of anti Siglec-F antibody in the lung of a mouse model of breast cancer and allergic asthma – Sima Shahmohammadi Farid

Poster 49 STAT6 AND SOCS3: Involvement in CCL26 Production by Bronchial Epithelial Cells in Asthma and It's Severity – Marie-Chantal Larose

Saturday, 22 July 2017

Session 7: Eosinophils in Clonal Diseases - Autoimmunity and Malignancy

Moderators: Moderators: Amy Klion, United States and Ariel Munitz, Isreal

8:00-8:30

State-of-the-Art: How can we stop eosinophils from taking over – lessons from HES trials – Amy Klion, United States

8:30-9:00

Cutting Edge: Bullous pemphigoid – Christian Sadik, Germany

9:00-9:30

Cutting Edge: Eosinophils in clonal T cell malignancies – Florence Roufosse, Belgium

9:30-9:45

Abstract Speaker: Eosinophils are an integral part of the tumor microenvironment in colorectal cancer exerting potent anti-tumorigenic activities – Hadar Reichman, Israel

9:45-10:00

Abstract Speaker: Evidence for a role of eosinophils in blister formation in bullous pemphigoid – Dagmar Simon, Switzerland

10:00-10:30 – Coffee Break

Session 8: Eosinophils, Glycobiology and Metabolism

Moderators: Christine Wennerås, Sweden and Elizabeth Jacobsen, United States

10:30-11:00

State-of-the-Art: Galectin-10 – the mysterious auto-crystalizing protein – Steven Ackerman, United States

Saturday, 22 July 2017

11:00-11:30
Cutting Edge: Eosinophil siglecs and their ligands – Bruce Bochner, United States

11:30-12:00
Cutting Edge: Eosinophils galectins and glycans – P. Sriramarao, United States

12:00-12:15
Abstract Speaker: Siglec-7 downregulates human eosinophil activation in vitro – Nadine Landolina, Israel

12:15-12:30
Abstract Speaker: Adipocytes support eosinophil migration and survival – Michal Itan, Isreal

12:30-14:00 – Conference Lunch, Meetings, Networking

Session 9: Working with Eosinophils: Tools and Biomarkers

Moderators: Bruce Bochner, United States and Josiane Neves, Brazil

14:00-14:30
State-of-the-Art: Laboratory techniques and resources for murine eosinophils – Elizabeth Jacobsen, United States

14:30-15:00
State-of-the-Art: Laboratory techniques and resources for human eosinophils – Lisa Spencer, United States

15:00-15:30
Cutting Edge: New mechanisms in IBD therapies – the mucosa under attack. What about the eosinophils? – Henit Yanai, Israel

15:30-15:45
Abstract Speaker: The role of autophagy in eosinophils – Nina Germic, Switzerland

15:45-16:00
Abstract Speaker: Haemosphere: A web-portal to delight immunologists and hematologists alike – Kirsten Fairfax, Australia

16:00-16:15 – Coffee Break

Battle of Eosinophilosophers – Is the Eosinophil a Good Guy or Bad Guy?

Moderator: Marie Carlson, Sweden

16:15-16:45
Good Guy Team: Seema Aceves, United States – **Bad Guy Team:** Allison Fryer, United States

A Memorial Congregation in Honor of Jamie Lee, PhD

Moderators: Helene Rosenberg, United States and Peter Weller, United States
Open to discussion from audience

16:45-17:30
Ehrlich Lectureship – In honor of Jamie Lee

19:00-23:00 – Conference Dinner and Boat Ride

Scientific Program

Sunday, 23 July 2017

Session 10: Eosinophilomics and Eosinophils Moving Forward

Moderators: Madeleine Rådinger, Sweden and Sergejs Berdnikovs, United States

9:00-9:30

State-of-the-Art: What I need to Know About Eosinophils and Transcription Factors – Patricia Fulkerson, United States

9:30-09:45

Abstract Speaker: Characterizing a leukocyte-specific RHO guanine nucleotide exchange factor proteoform in eosinophils – Keren Turton, United States

09:45-10:15 – Coffee Break

Moderators: Florence Rofousse, Belgium

10:15-11:00

Panel Discussion: Where we were, where are we now, and where we are going – Per Venge, Sweden, Andrew Wardlaw, United Kingdom, Seema Aceves, United States and Helene Rosenberg, United States

11:00-12:00

Acknowledgements, Final Awards and Adjournment – Marie Carlson, Sweden and Christine Wennerås, Sweden

Gleich Award

Recipient of the 2017 Gleich Award

Dr. Claire Mesnil will receive the fourth Gerald J. Gleich prize to be awarded at the 10th Biennial Symposium of the International Eosinophil Society, Inc. in Gothenburg, Sweden. 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.

LUNG-RESIDENT EOSINOPHILS REPRESENT A DISTINCT REGULATORY EOSINOPHIL SUBSET

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THE GERALD J. GLEICH AWARD
INTERNATIONAL EOSINOPHIL SOCIETY, INC.
2017

Claire Mesnil

In recognition of the most intriguing, high impact finding related to eosinophil biology published in the years 2015-2017.

International Eosinophil Society
IES

A Memorial Congregation in Honor of Jamie Lee, PhD

Recipient of the 2017 Ehrlich Lectureship

**as printed in April JACI*

In lasting tribute: Dr. James J. Lee (1958-2017)

It is with deep sorrow that we report the untimely death of our friend and colleague Dr. James (Jamie) Lee, who passed away on Saturday, March 25, 2017, at his home in Scottsdale, Arizona.

Originally from Long Island, New York, Dr. Lee received a BS in Biochemistry from the State University of New York at Stony Brook. After graduate studies with Dr .Eric Davidson at Caltech and postdoctoral studies at Columbia University, he joined the staff at Mayo Clinic Arizona, where he held the position of Professor of Biochemistry and Molecular Biology in the Division of Pulmonary Medicine. In collaboration with his wife and lifelong research partner, Dr. Nancy A. Lee, the long-term focus of Dr. Lee's work has been improving our understanding of how eosinophilic leukocytes contribute to the pathogenesis of allergy, asthma, and cancer.

Dr. Lee has led by example in being a strong advocate of collaboration and service to the scientific community. Without doubt, among Dr. Lee's finest contributions to eosinophil biology are a unique series of transgenic and gene-deleted mice. The first of these mice, NJ.1638, include the IL-5 transgene expressed under the control of the T-cell CD3d promoter that results in massive systemic eosinophilia. The Lee laboratories also created TgPHIL and iPHIL, both eosinophil-deficient models that use the eosinophil peroxidase promoter to facilitate lineage depletion. Use of these mouse models in the Lee laboratories and many others have transformed our understanding of the importance of eosinophils in a range of immune responses. Dr. Lee was particularly interested in the concept of eosinophils as key mediators of immune regulation and tissue remodeling. Most recently, the Lees and colleagues introduced eoCre, which also uses the eosinophil peroxidase gene promoter to direct expression of Cre recombinase, resulting in a means to generate eosinophil-specific gene deletions. Together with his development of gold-standard reagents, such as antibodies recognizing eosinophil granule proteins, eosinophil biology has grown and flourished in no small part because of the generosity of the Lee laboratories.

Dr. Lee has held many important posts and responsibilities, including past President of the International Eosinophil Society, where he also served on the Board of Directors. He was a member of the National Institutes of Health Taskforce on the Research Needs of Eosinophil-Associated Diseases and served as Co-Editor in Chief for the multi-author text Eosinophils in health and disease (Elsevier, 2012).

Dr. Lee was scheduled to receive the 2017 Paul Ehrlich Award this coming July, the highest award bestowed on a colleague who has ".made seminal scientific contributions to research on the eosinophil and related allergy/immunology fields." by the members of the International Eosinophil Society.

It is difficult to convey fully the effect of the loss of a member of our close-knit research family. We will always remember Jamie, and we will be inspired by his exuberant sense of humor, boundless energy, extraordinary creativity, and unwavering commitment to the scientific community.

*Helen F. Rosenberg, MD, PhD
Elizabeth A. Jacobson, PhD
Bruce Bochner, MD
Peter F. Weller, MD

PAUL EHRLICH LECTURESHIP
INTERNATIONAL EOSINOPHIL SOCIETY, INC.
2017

James J. Lee, MD

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

International Eosinophil Society
IES

Service Award

Recipient of the 2017 International Eosinophil Society Service Award

The International Eosinophil Society, Inc., its leaders and members present to Dr. Peter F. Weller 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.

Peter F. Weller, M.D., MACP, the William Bosworth Castle Professor of Medicine at Harvard Medical School, is Chief of both the Division of Infectious Diseases and the Division of Allergy and Inflammation at Beth Israel Deaconess Medical Center, Harvard Medical School. He is also a Professor of Immunology and Infectious Diseases at the Harvard Chan School of Public Health. Dr. Weller received his M.D. from Harvard Medical School and completed his medicine training at the Peter Bent Brigham Hospital, his infectious diseases training at the Laboratory of Parasitic Diseases, National Institutes of Health and at Massachusetts General Hospital, and his allergy training at the Brigham and Women's Hospital. He is board-certified in Internal Medicine, Infectious Diseases and Allergy and Immunology.

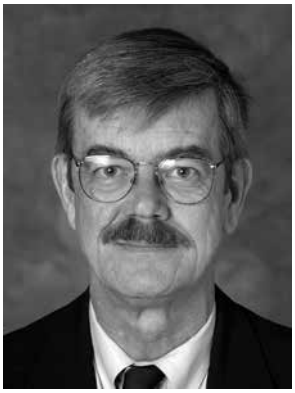
Dr. Weller's research studies, supported by longstanding NIH grants, have focused on delineating mechanisms of leukocyte functioning, including a major focus on the immunobiology of eosinophilic leukocytes. Of his over three dozen trainees, most have senior academic positions nationally and internationally. Dr. Weller's clinical areas of interest and expertise include parasitic infectious diseases and allergic diseases, including eosinophil-associated syndromes and diseases. In these areas, he has a record of continuing contributions, including over 450 publications.

With Dr. Weller's career long interest in eosinophils, he along with others, including the late Redwan Moqbel, shepherded the coalesce of common eosinophil interests amongst our international community to lead to the formation of the International Eosinophil Society and its maturation into fully active Society. Dr. Weller has served as the Secretary-Treasurer of the International Eosinophil Society and currently is the President of the Society.

SERVICE AWARD
INTERNATIONAL EOSINOPHIL SOCIETY, INC.
2017

Peter F. Weller, MD

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



EOSINOPHILS, PATHOGENS, AND INFECTIOUS DISEASE: NEW PERSPECTIVES

Helene F. Rosenberg[1]

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Paul Ehrlich (1854-1915) is credited with recognizing eosinophils as a distinct leukocyte population and for devising an original method for staining them *ex vivo*. We now recognize eosinophils as cells that develop in the bone marrow from pluripotent progenitors that are released into the systemic circulation and ultimately migrate to peripheral tissues. While it is clear that eosinophils can and do respond to pathogens, the nature and outcome of these interactions are not fully understood.

Among earliest observations, eosinophils are recruited in large numbers into peripheral blood and peripheral tissues in association with helminth infection, notably *Schistosoma mansoni*. Studies of eosinophil-parasite interactions in tissue together with experiments demonstrating eosinophil-mediated larval killing *ex vivo* suggested that eosinophils contributed to primary host defense. However, studies carried out in cytokine and eosinophil-depleted and deficient mouse models did not fully support these conclusions. Moreover, recent work the nematode *Trichinella spiralis* suggested that eosinophils can also serve the needs of the parasite, in this case, promoting larval growth in skeletal muscle via specific immunomodulatory mechanisms. The role(s) of eosinophils in helminth infection are clearly complex, and go far beyond simple interactions between eosinophils and target pathogens.

Similarly, eosinophils recruited to the respiratory tract in response to allergens and Th2 cytokines have been implicated in limiting virus replication and in subverting the lethal sequelae of respiratory virus disease. Eosinophil-mediated antiviral mechanisms have included accelerated virion clearance, virus-mediated eosinophil degranulation and recruitment of antiviral CD8+ T cells. Clearly the eosinophil-mediated antiviral mechanisms are similarly complex, multifactorial and include features beyond simply interactions between eosinophils and pathogens.

Eosinophils have also been implicated in diseases involving *Pneumocystis jirovecii*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and other fungal pathogens, likewise via complex mechanisms involving host targets.

Eosinophils also interact with bacteria. This is a most intriguing topic, given predominance of eosinophils in gastrointestinal tract at homeostasis. While cationic granule proteins are bactericidal in studies performed *ex vivo*, eosinophils are not typically recruited to systemic circulation and neutrophils predominate in bacterial-enriched lesions in tissues.

Nonetheless, hyper-eosinophilic IL5tg mice are protected against gram-negative sepsis and, and eosinophils respond to bacteria by releasing granule proteins and DNA. Recent evidence suggests that eosinophils may may modulate the nature and contents of the gut microbiome and likewise serve as a crucial barrier against infection with the gram-negative pathogen, *Clostridium difficile*.

In order to explore and to understand the role(s) of eosinophils in infectious disease, we need to know more about the ways in which eosinophils sense change and respond to their environments. First and foremost, the majority of evidence to date suggests that eosinophils are primarily immunomodulatory cells. Eosinophils are clearly able to interact with pathogens and sense their presence. However, via release of cytokines, granule proteins and lipid mediators, eosinophils may have more of an impact on disease via their ability to modulate responses of epithelial cells, endothelial cells, fibroblasts and other leukocyte populations in the local microenvironment. As such, eosinophils may promote host defense by promoting immunomodulatory interventions that limit disease pathogenesis.

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EOSINOPHILS AND *ASPERGILLUS FUMIGATUS* INFECTION: A DNA TRAP RELEASE STORY

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The release of extracellular DNA traps (ETs) by leukocytes has been described as an innate immune response mechanism that is relevant in many disorders including fungal diseases. Different stimuli induce human eosinophil ETs (EETs) release. *Aspergillus fumigatus (A. fumigatus)* is an opportunistic fungus that may cause eosinophilic allergic bronchopulmonary aspergillosis (ABPA). It has been reported that *e*osinophils are important to the clearance of *A. fumigatus* in infected mice lungs. However, the immunological mechanisms that underlie the molecular interactions between *A. fumigatus* and eosinophils are poorly understood. We investigat-

ed the presence of EETs in the bronchial mucus plugs of ABPA patients. We also determined whether *A. fumigatus*-induced human eosinophils EETs release *in vitro*. We identified abundant nuclear histone-bearing EETs in the bronchial secretions obtained from ABPA patients. *In vitro,* we demonstrated that *A. fumigatus* induces EETs release through a mechanism independent of reactive oxygen species but associated to eosinophil death, histone citrullination, CD11b and the Syk tyrosine kinase pathway. Our studies may contribute to the understanding of how eosinophils recognize and act as immune cells in response to *A. fumigatus*, which may lead to novel insights regarding ABPA patient treatment.

EOSINOPHILS REGULATE HOMEOSTASIS OF THE GASTROINTESTINAL TRACT

Derek M. McKay, PhD

Professor, Gastrointestinal Research Group, Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

Infection with helminth parasites is a potent stimulus of host immunity and mobilizes eosinophils. The role of eosinophils following infection with helminths in far from clear. Infection with a variety of helminths, including the rat tapeworm, *Hymenolepis diminuta*, can reduce the severity of concomitant disease. We find that mice infected with *H. diminuta* 8 days prior to intra-rectal administration of oxazolone had more severity colitis than oxazolone-only treated mice, and that this correlated with increased numbers of eosinophils in the colon. Use of adenovirus expressing IL-5, IL-5 neutralizing antibodies and eotaxin-1 and -2 knockout mice support the view that the exaggerated disease in the *H. diminuta*-infected mice was mediated via eosinophils, despite the increased capacity of splenocytes to produce the immunoregulatory IL-10. Oxazolone-colitis in mice bears similarity to human ulcerative colitis, and the latter can be characterized by an eosinophilia. Inflammatory bowel disease is accompanied by increases in epithelial permeability. Observations in colonic biopsies from patients with ulcerative colitis and *in vitro* studies with mast cells, eosinophils and the human-colon derived T84 epithelial cell line indicate that cholinergic stimulation of eosinophils results in increased epithelial permeability to macromolecules via activation of mast cells. Finally, apoptotic eosinophils in conjunction with type-II cytokines may be an additional stimulus for the induction of regulatory, pro-resolution macrophages. Despite the identification of eosinophils and documentation of these cells in the gut under defined allergic-type conditions and following infection with helminth parasites, the cell remains enigmatic: it is emerging that eosinophils, like all immune cells, are contextually defined and can be a pivotal influence in gut homeostasis directing normal physiological reactions or driving pathophysiological responses.

THE HUMAN INTESTINAL MICROBIOME

Ingegerd Adlerberth

The gut microbiome represents the most complex bacterial community of the human body. The large intestine of an adult individual hosts a tremendous variety of microbes, including several hundred of different bacterial species, as well as some archeal and yeast species. Most bacteria residing in the large intestine are strictly anaerobic, and around half of these species have not yet been cultured. Most gut bacterial species belong to the phyla Firmicutes, Bacteroides, Actinobacteria, Fusobacteria and Proteobacteria. Major anaerobic bacterial genera include e.g. *Faecalibacterium, Eubacterium, Ruminococcus, Clostridium, Coprococcus, Blautia, Anaerostipes, Roseburia, Bacteroides, Parabacteroides, Alistipes, Bifidobacterium* and *Collinsella*. Facultatively anaerobic bacteria are present at lower concentrations than the anaerobes. Common facultative bacteria include *Escherichia coli* and other enterobacteria, enterococci and streptococci. The gut microbiome is formed through the successive establishment of different bacteria in infancy and early childhood.

The gut microbiome has a number of effects on the host. Not the least, interactions between gut bacteria and the gut-associated immune system are of great importance. Such interactions may influence the maturation of immunological tolerance mechanisms in infancy and early childhood, which, in turn, may have implications for protection from allergy and other immunoregulatory disorders.

The presentation will give an overview of the composition of the gut microbiome, and briefly discuss its effects on immune development.

STATE-OF-THE-ART: EOSINOPHILS IN IBD – HEALERS OR DESTROYERS?

Marie Carlson

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The inflammatory bowel disease

The concept of inflammatory bowel disease (IBD) comprises the disorders Crohn's disease (CD), ulcerative colitis (UC) and the microscopic colitis types lymphocytic colitis and collagenous colitis (CC). The aetiology of the diseases is unknown, but they are characterized by chronic inflammation. IBD usually evinces intermittent aggravation, but sometimes the inflammation is chronically active. Patients with CD and UC suffer from frequent diarrhea, blood in stool, abdominal pain, and weight loss. Incidence has increased in the Western world in recent years, and new cases occur primarily in the group between 20-40 years. Symptoms of CC are primarily frequent diarrhea and the age of onset is 50-70 years, with women over-represented.

Under normal conditions, the intestinal mucosa is in a state of "controlled inflammation". Different subsets of T-cells are present in the healthy intestine, as are eosinophils and antigen-presenting cells. However, there is a predominance of anti-inflammatory and regulatory cytokine responses that keep the mucosal homeostasis intact. In IBD, the balance between mucosal responsiveness and tolerance towards antigens is disturbed, and there is an exaggerated immune response to the commensal flora in genetically predisposed persons.

The eosinophil granulocyte in IBD

It is well established that the neutrophil granulocyte accumulates and infiltrates in the local process in IBD. In recent years more attention has been paid to the eosinophil granulocytes, which are often seen in the histological picture in IBD. We have demonstrated that there is increased and selective intraluminal release of the eosinophil granule proteins in patients with UC, which indicates that eosinophils may be an important part of the pathophysiological mechanism in UC. Also demonstrated increased numbers of activated eosinophils in colon biopsy samples from patients with active UC and CD as well as quiescent UC. The levels of eosinophil-derived granule proteins correlated with disease severity, indicating a pro-inflammatory role, but the presence of activated eosinophils in the absence of inflammation suggests that eosinophils may be involved in tissue repair or in the development of fibrosis in quiescent UC. In a recently published paper, we have shown that eosinophils also play a crucial pathophysiological role in CC.

There has long been a demand for simple, objective and reliable tools for the assessment of mucosal inflammation to replace or complement colonoscopy in the diagnosis and monitoring of IBD patients. To measure inflammation markers in feces has proven to be both simple and convenient for patients. In remission, a dramatic drop in eosinophil markers in feces and good correlation with clinical and endoscopic scores and PAD were observed. In a new study we demonstrate that fecal ECP is useful in monitoring disease activity and treatment outcome in patients with CC, and eosinophil fecal makers where superior to rectal NO and locally collected fluids using the patch technique for the measurement of inflammation markers in patients whit active UC. In an ongoing longitudinal study including 112 patients with IBD, we will evaluate if a relapse can be predicted based on eosinophil fecal markers. Each individual leaves a feces sample every three months and during relapses of the disease in the course of two years. The presentation will provide preliminary data from this study and an overview of the field.

PATHOGENESIS OF EOSINOPHILIC ESOPHAGITIS

Marc E. Rothenberg, MD, PhD

Herein I will review the molecular genetic, and cellular bases of eosinophilic esophagitis (EoE) and their implications for emerging therapeutics and diagnostics. EoE was historically distinguished from gastroesophageal reflux disease on the basis of histology and lack of responsiveness to acid suppressive therapy, but it is now appreciated that esophageal eosinophilia can respond to proton pump inhibitors. Genetic and environmental factors contribute to risk for EoE—particularly early-life events as assessed by twin and microbiome studies. Disease pathogenesis involves activation of epithelial inflammatory pathways (production of eotaxin-3 [encoded by *CCL26*]), impaired barrier function (mediated by loss of desmoglein-1), increased production and/or activity of transforming growth factor-β, and induction of allergic inflammation by eosinophils and mast cells. Susceptibility has been associated with variants at 5q22 (*TSLP*) and 2p23 (*CAPN14*), indicating roles for allergic sensitization and esophageal specific protease pathways. There is a central role for innate epithelial responses regulated by esophageal specific caplain-14 based on genetic and functional data. Loss of esophageal epithelial differentiation (tissue identity) underlies the disease pathophysiology. Mendelian disorders, involving single gene mutations (*DSG1* and *SPINK5*) provide proof-of-concept for the importance of homeostatic barrier and proteases in protecting the esophagus from allergic inflammation. Evidence for the involvement of food antigen driven effector memory Th2 cells and their associated cytokines will be presented. I will leave the message that EoE is a unique disease characterized by food hypersensitivity, strong hereditability influenced by early-life exposures, disruption of the balance between esophageal specific proteases and anti-proteases, and allergic inflammation and that the disease is remitted by disrupting inflammatory and T-helper type 2 cytokine–mediated responses (including anti-IL-13) and through dietary elimination therapy.

THE EOSINOPHIL IN EOSINOPHILIC ESOPHAGITIS: THE "CELL-IN-CHIEF" OR SIMPLY AN "ASSISTANT"?

Alex Straumann, Carine Blanchard, Christian Bussmann, Susanne Hoesli, Petr Hruz, Evelyne Kozlowski, Katia Safroneeva, Alain M. Schoepfer, Dagmar Simon,[8] Mirjam Tschanz, and Hans-Uwe Simon from the Swiss EoE Research Network

Background Eosinophilic Esophagitis (EoE) is a chronic-inflammatory disease of the esophagus defined clinically by symptoms of esophageal dysfunction and histo-pathologically by an eosinophil-predominant infiltration. EoE has a strong genetic component. We identified in four EoE-families totally five members with an "EoE-like Syndrome", presenting with typical symptoms of EoE responding promptly to treatment with topical corticosteroids, but without tissue eosinophilia. We therefore investigated this intriguing syndrome of "EoE without Eosinophilia", in order to improve the understanding of this emerging inflammatory condition.

Methods The five patients suffering from "EoE-like Syndrome" were evaluated by laboratory analyses, endoscopy, histologic and

quantitative immuno-histologic examinations and genome-wide association analyses. In addition, we searched in all 46 members of these EoE-families for EoE-associated molecular abnormalities.

Using immunohistochemistry we detected in the esophagus of patients with "EoE-like Syndrome" a chronic, Th2 type inflammation, but definitely a lack of eosinophils. In addition, we found the EoE-risk allele *TSLP rs3806932* in their genome. First generation offspring of EoE-like syndrome patients had on average a 40% risk of being affected by conventional EoE.

Results These five members of EoE families suffering from "EoE without eosinophilia" do formally not fulfill the diagnostic criteria of EoE. However, clinical manifestation, the finding of a Th2 type inflammation, the bequeath of conventional EoE to their offspring and the detection of the EoE-risk allele *TSLP rs3806932* in their genome strongly suggests a uniform underlying pathogenesis.

Conclusions Conventional EoE with the predominant eosinophilia might therefore be only one phenotype of this dysphagia syndrome. The role of the eosinophils and the disease-definition must therefore be reconsidered. Moreover, as these patients have endoscopically and histologically only discreet abnormalities; this syndrome might so far often be misdiagnosed as functional dysphagia.

EOSINOPHIL EFFECTOR FUNCTIONS

Peter F. Weller, MD, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston MA USA

Eosinophils are endowed with a multitude of means by which these cells can exert "effector" functions. The eosinophil's distinct content of their granule-contained cationic proteins, including MBP, ECP and EPX, provide one means for eosinophils to exert effector functions. The capacities of eosinophils to elaborate lipid mediators, such as leukotriene C_4, provide additional paracrine effector mechanisms.

In addition, eosinophils are increasingly recognized as potential sources of diverse cytokines and chemokines that contribute to their "effector" roles in innate and adaptive immunity. The recognitions of eosinophils as sources of cytokines derive from both studies in mice and in humans. We consider issues that underlie the capabilities of eosinophils to selectively secrete cytokines and to mediate eosinophil effector functions in innate and adaptive immune responses.

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MOLECULAR MECHANISMS REGULATING EOSINOPHIL GRANULE PROTEIN RELEASE AND TOXICITY

Hans-Uwe Simon

Institute of Pharmacology, University of Bern, Bern, Switzerland.

Eosinophils are white blood cells that function in innate immunity and participate in the pathogenesis of various inflammatory and neoplastic disorders. Their secretory granules contain four cytotoxic proteins, including the eosinophil major basic protein (MBP-1). How MBP-1 toxicity is controlled within the eosinophil itself yet activated upon extracellular release is unknown. Here we show how intragranular MBP-1 nanocrystals restrain toxicity enabling its safe storage and characterize them with an x-ray free electron laser. Following eosinophil activation, MBP-1 toxicity is triggered by granule acidification followed by extracellular aggregation which mediates damage to pathogens and host cells. Larger non-toxic amyloid plaques are also present in tissues of eosinophilic patients, in a feedback mechanism which likely limits tissue damage under pathological conditions of MBP-1 oversecretion. Our results suggest that MBP-1 aggregation is important for innate immunity and immunopathology mediated by eosinophils and clarify how its polymorphic self-association pathways regulate toxicity intra- and extracellularly.

CHECKPOINT INHIBITORY RECEPTORS ON EOSINOPHILS

Ariel Munitz[1]

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Eosinophil differentiation, migration and activation are tightly regulated by a balance of activation and inhibitory signals. In fact, eosinophils express numerous receptors containing immunoreceptor tyrosine-based activation or inhibitory motifs (ITAMs and ITIMs, respectively). These receptors can regulate signals that are initiated by cytokines and chemokines, growth factors and even innate immune components. Therefore, such receptors have key roles in various cellular and pathological responses of eosinophils and are thus potential targets for future therapeutics. One of the best examples for targeting ITIM-bearing receptors as therapeutic targets comes from the field of immune oncology where targeting CTLA-4, PDL-1 and PD-1 on cytotoxic T cells has substantial therapeutic benefit and is now collectively termed "checkpoint inhibitors". In this session, we will outline our new data regarding the anti-tumorigenic activities of eosinophils in colorectal cancer. Furthermore, we will describe the role of ITIM-bearing receptors such as PIR-B and CD300f in the regulation of eosinophil migration and activation in the GI tract. In particular, we will exemplify the potential to target such receptors on eosinophils in cancer as a new therapeutic strategy aimed at unleashing anti-tumorigenic activities of eosinophils.

EOSINOPHILS IN ASTHMA

Andrew Wardlaw

The close association between eosinophils and asthma has been recognised for many decades. Concepts regarding the role of eosinophils in asthma have fluctuated, ranging from being the final effector cell responsible for all aspects of asthma through bystander cells of no consequence to being responsible for an amelioration of the inflammatory process. Asthma is a common heterogeneous condition that presents with variable expression of four relatively distinct pathophysiological abnormalities. These are i) rapidly variable airflow obstruction due to hyperresponsiveness of the airway smooth muscle (ASM) to bronchoconstrictor stimuli, ii) airway inflammation which is usually but not invariably eosinophilic, iii) a heightened cough reflex and iv) tissue damage, reflected mainly in fixed airflow obstruction and bronchiectasis which is usually a feature of more severe disease. Clinically the ASM hyperresponsiveness presents as chest tightness, wheeze and episodic shortness of breath whereas the inflammatory component presents as bronchodilator resistant exacerbations, which can lead to hospital admission and even death. In the majority of people with asthma the inflammation drives the airway hyperresponsiveness, but they can occur independently. The inflammation in about 80% of cases is caused by a T2 response to inhaled allergens which include dust mites, animal danders, pollens and fungal spores. The resultant eosinophilic inflammation is highly responsive to corticosteroids which when given as inhaled topical treatment is together with beta 2 agonists the mainstay of management. While the majority of patients can be controlled with modest amounts of inhaled therapy about 5% of asthmatics have more severe and difficult to control disease. In many cases this is due to poor compliance with therapy, but in some people the inflammatory process is difficult to control. Over the last twenty years there has been considerable investment in what can broadly be described as anti-Th2 therapies, which have sought to inhibit the 'allergic' limb of the immune response. The first of these omalizumab licensed about 15 years ago has been a successful therapy for severe atopic asthma. More recently several other antibodies targeted at the Th2 pathway and particularly eosinophils have been developed and are now reaching the clinic. The development of these treatments has shed considerable light onto what part T2 inflammation in general and eosinophils in particular play in asthma.

The first drug to be developed was mepolizumab by GSK. This biological therapy binds to IL-5 and prevents it binding to its receptor. It is therefore very successful at reducing blood eosinophil counts in most eosinophilic conditions. Initial experience with this drug was disappointing in that it failed to block the allergen challenge response or improve FEV_1 or day-to-day symptoms in moderate asthma. However we had hypothesized that eosinophils primary role was in causing exacerbations of asthma rather than day-to-day symptoms which are more closely linked to variable airflow obstruction. We had also made the observation that only a proportion of asthmatics had active eosinophilic inflammation at the time of assessment. When we treated severe eosinophilic asthma with mepolizumab using exacerbations as a primary outcome there was about a 50% reduction in severe exacerbations. This was replicated in phase 3 trials and led to the licensing of mepolizumab (Nucala) for severe eosinophilic asthma in the USA and Europe in late 2015. Close on the heels has been the development of reslizumab by TEVA. This biological therapy which also binds IL-5 was initially created by Schering Plough in the 1990s, but was not pursued when initial trials similar in design to the first GSK studies were disappointing. TEVA followed a similar development path with reslizumab to that adopted by GSK in the later development of mepolizumab and were also able to demonstrate about a 50% reduction in exacerbations in eosinophilic asthma. They also found a modest improvement in FEV_1, but this was probably due to subtle differences in study design rather than any difference in efficacy. Reslizumab (CINQAIR) was licensed in the USA in March 2016, although unlike Nuclera it has not yet received approval for prescribing in the UK. Lastly benralizumab is a biological therapy developed by Astra Zeneca/MedImmune which binds to the IL-5 receptor and causes antibody dependent cytotoxicity of eosinophils and basophils. It also efficiently ablates eosinophil counts in the blood. Phase 3 trials for benralizumab were reported in the autumn of 2016 and applications for licensing in Europe and the USA are under review. As with mepolizumab and reslizumab there was a 50% reduction in severe exacerbations and a modest (<200ml) improvement in FEV_1. Biological therapies aimed at the other cytokines involved in the T2 immune response are at various stages of development. The efficacy of these is still emerging, but it appears that they also result in about a 50% reduction in exacerbations in eosinophilic asthma suggesting a linear pathway from initiating cytokines such as IL-33 and TSLP through T2 cell derived IL-4 and IL-13 and then to IL-5 as the final effector stage mediated by eosinophils. In this model eosinophils are largely responsible for the majority of the negative effects associated with T2 inflammation in asthma. Omalizumab also appears to act on this pathway, although the precise point at which they act is still not clear.

A number of conclusions can be drawn from these clinical trials and other supporting studies:

- Eosinophilic (T2) inflammation is found in most people with asthma, particularly in its more severe manifestations or during periods of poor control. Some asthmatics, mainly those with mild disease or isolated airway smooth muscle dysfunction, do not have evidence of granulocyte inflammation, but do have evidence of mast cell infiltration of the airway smooth muscle. In about 10% of asthmatics recurrent bacterial bronchitis associated with neutrophilic inflammation appears to drive their disease.
- Eosinophils are neither necessary nor sufficient to cause airway smooth muscle hyperresponsiveness which is the abnormality which underlies variable airflow obstruction and the day-to-day symptoms of asthma. Their importance in causing this hallmark of asthma is very questionable.

- The benefits of prednisolone in asthma are in large part due to their ability to suppress eosinophilic inflammation.
- Eosinophilic inflammation is a feature of about 50% of severe exacerbations requiring oral prednisolone and probably a much larger percentage of life threatening asthma exacerbations requiring hospitalization and which can lead to death.
- The peripheral blood eosinophil count is an adequate marker of the presence of eosinophilic inflammation and the likelihood of exacerbations. A count of $>0.3X10^9$/L appears a pragmatic cut off point for guiding response to anti-T2 biological therapy.
- There is an exacerbation prone phenotype in asthma characterized by a marked eosinophilia in the blood and airways. This endotype of 'hypereosinophilic' asthma which is often a feature of adult onset disease is particularly responsive to anti-eosinophil biological therapy
- The mechanism by which eosinophils cause severe exacerbations is not understood. It is striking that the 100mg dose of Nucala which is the dose chosen by GSK for commercial treatment of asthma, reduced exacerbations without completely suppressing the sputum eosinophilia.
- The cause of the eosinophilic inflammation in adult onset non-atopic asthma is not known.
- In a large percentage of people with asthma with active eosinophilic inflammation poor control is due to sub-optimal adherence to inhaled corticosteroids. However a proportion do have inflammation which is not suppressed by inhaled corticosteroids although it is effectively suppressed by systemic corticosteroids and anti-eosinophil biological therapies
- Corticosteroids resistance at a molecular level, even in severe asthma, is very rare.
- Partial ablation of eosinophils using biological therapy does not appear to be associated with any major adverse events.

In summary it is now clear from the use of specific anti-eosinophil biological therapies that eosinophils play a specific role in asthma as a cause of severe exacerbations, particularly those that lead to hospitalization and death. The development of these safe and effective treatments represents a major advance in the management of the endotype of severe, eosinophilic, exacerbation prone asthma. It is also a proof of concept to encourage the development of specific anti-eosinophil therapies which will be cheaper and orally bio- available and therefore can be used to treat a wider group of people with a lower risk of exacerbations who nonetheless make up 50% of asthmatics who die from their disease.

ROLE OF GROUP 2 INNATE LYMPHOID CELLS IN EOSINOPHILIC ASTHMA

Roma Sehmi

Asthma is a chronic disease characterized by inflammation of the airways, reversible airflow obstruction and airway hyperresponsiveness (AHR). It can be classified into various endotypes, the most clearly described being eosinophilic asthma. Eosinophils are a major contributor to physiological changes and re-modeling in asthma. Treatments that control airway eosinophilia are associated with a decrease in symptoms and exacerbation rates. Investigating {Sehmi, 2009 #829@@author-year}{Sehmi, 2009 #829} mechanism(s) that drive airway eosinophilia will provide novel drug targets that can effectively control airway eosinophilia and have steroid sparing effects.

Airway eosinophilia arises 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 namely IL-5.{Sehmi, 2009 #829@@author-year}{Sehmi, 2009 #829} In addition, other type 2 cytokines, IL-4 and IL-13, can prime the migration of EoPs thereby promoting the exaggerated eosinophilopoietic environment observed in the airways of asthmatics. Understanding pathobiological pathways that promote local production of type 2 cytokines may provide effective targets for controlling eosinophilic asthma particularly in those patients refractory to the gold-standard of treatment, corticosteroids.

A newly described group of non-B, non-T cells lacking antigen recognition receptors, have been termed group 2 innate lymphoid cells (ILC2). The cells have been shown to produce more canonical type 2 cytokines (IL-5 and IL-13), on a cell per cell basis, than CD4+ T lymphocytes. Genome wide association studies have found a close relationship between the incidence of asthma and single nucleotide polymorphisms (SNPs) in several genes including (i) alarmin cytokines that can activate ILC2s - *IL1RL1 (IL-33 receptor, ST2), IL18R, IL33, TSLP* and (ii) *RORα* – a key transcription factor for ILC2 development. These findings indicate that epithelial-derived cytokines and ILC2s are central players in the pathobiology of asthma. This has resulted in a paradigm shift that asthma is not simply a T helper 2 cell-dependent, IgE-mediated allergic inflammatory disease but involves an innate pathway where ILC2s can provide a source of type 2 cytokines likely critical for initiation of adaptive type 2 immune responses. Evidence for a role for ILC2s in eosinophilic asthma has come from murine studies. However, recent studies have reported increased numbers of ILC2s in the blood and airways of asthmatics and these numbers correlated with disease severity. Interestingly, the greatest number of activated ILC2s were found in the sputum of severe asthmatics with uncontrolled eosinophilia despite high dose oral steroid therapy, suggesting putative steroid-resistance of this class of cells. In addition, ILC2s appear to be an early source of type 2 cytokine producing cells in the airways of mild asthmatics following inhaled allergen challenge. Minimal systemic expansion was observed indicating a localized expansion and activation of ILC2s at mucosal surfaces in eosinophilic asthmatic responses. Targeting local activation of ILC2s in asthmatic airways may provide a critical control for modulating uncontrolled eosinophilic inflammation.

Invited Speaker Abstracts

ACTIVATE INHIBITION OR INHIBIT ACTIVATION OF MAST CELL / EOSINOPHIL ALLERGIC EFFECTOR UNIT TO TREAT ALLERGIC DISEASES

Francesca Levi-Schaffer, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Allergic inflammation dominant cells are the mast cells and the eosinophils. Mast cells as activated by IgE mechanisms via allergens are the recognized primum movens while eosinophils infiltration and persistence in the inflamed tissue with the mast cells is the accepted feature of the late stage and of the chronic outcome of allergy.

Over the years we have defined a pro-inflammatory cross-talk between these two cells that we have named the Allergic Effector Unit (AEU). Mast cell/eosinophils interactions that result in increased eosinophils chemotaxis, survival, degranulation, cytokine production and in mast cell survival, IgE-dependent and independent degranulation and cytokine production are mediated by both released mediators (soluble interactions) and by receptor/ligands binding (physical interactions).

Prominent physical players of the activating AEU are the two receptors/co-receptors/ligands CD48 and 2B4. Nevertheless we have also described the presence and functional activity of two inhibitory receptors, i.e. CD300a and Siglec-7 on mast cells and on eosinophils that can indicate an anti-inflammatory or even pro-resolution activity within the AEU.

The goal of our research it to define in the framework of the AEU potential new targets for immunopharmacological intervention in allergic diseases. Our studies have been carried out *in vitro* on both human and murine mast cells and eosinophils and *in vivo* studies in murine models of allergic peritonitis, atopic dermatitis, allergic asthma.

In my presentation I will therefore illustrate our findings related to CD48/2b4 as expressed by mast cells and eosinophils as a main activating receptor bridging allergy and *S. aureus* infection that always co-exist in allergic tissues. I will moreover show our results on the allergic reactions inhibitory properties of CD300a and Siglec-7 as main inhibitory receptors as expressed by mast cells and eosinophils. Thus our strategy to treat allergy by inhibiting activation and/or by activating inhibition of mast cells, eosinophils and the AEU will be discussed in the framework of some data obtained in human allergic diseases.

EOSINOPHILS AS MASTERS OF T CELL FATE

Christine Wennerås, Department of Infectious Diseases, Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden

Eosinophilic granulocytes are associated with parasitic infections and allergic diseases, but their precise functions in these conditions are poorly defined. During the last decades, an increasing number of studies have indicated that eosinophils can regulate the development as well as the functions of T cells and their subsets in human beings and in mice. Thymic eosinophils may have a role in the negative selection of thymocytes in mice. Moqbel et al demonstrated the capacity of blood eosinophils from atopic human donors to selectively inhibit the proliferation of Th1, but not Th2 cell lines, through the catabolism of tryptophan. The same group proposed that human thymic eosinophils may similarly polarize thymocyte development toward Th2 responses. In contrast, eosinophils were recently shown to suppress Th2 responses in the Peyer's patches of parasite-infected mice. Moreover, murine small intestinal eosinophils downregulate TH17 cells under steady-state conditions by the constitutive secretion of IL-1 receptor antagonist. Lung eosinophils are apparently needed for the recruitment of effector T cells into the airways in murine models of pulmonary allergy. Our group has shown that human eosinophils suppress polyclonal T cell proliferation in a cell-contact dependent manner that is partially mediated through galectin-10. Furthermore, we have identified a subpopulation of eosinophils in the blood of healthy individuals that are potent suppressors of T cell proliferation that we have designated "suppressive eosinophils". To conclude, new functions of eosinophils are being revealed both regarding their capacity to regulate T cell homeostasis and to control T cell activities in various disease states.

Cutting Edge – Bullous Pemphigoid

Bullous pemphigoid (BP) is the most common disease of the group of pemphigoid diseases. These diseases have in common that they are caused by autoantibodies directed to structural proteins of the dermal-epidermal adhesion complex of the skin and mucous membranes. These autoantibodies deposit at the dermal-epidermal junction and induce the recruitment of granulocytes into the dermis. Subsequently, the dermal-epidermal adhesion complex is degraded and a subepidermal cleft is formed, which clinically presents as skin blisters and erosions.

The most striking histopathological feature of BP is dominance of eosinophils inflamed skin of BP patients. In addition, BP patients often present with eosinophilia. These features of the disease let a significant contribution of eosinophils to the pathogenesis of BP appear likely. However, results from experimental models on a putative role of eosinophils have been contradictory and therapeutic strategies inhibiting eosinophils did not deliver convincing results. Thus, the role of eosinophils in BP has been under debate for decades. In the Cutting Edge Lecture *Bullous Pemphigoid*, Prof. Christian Sadik (University of Lübeck, Germany) will summarize and discuss the arguments for and against a role of eosinophils in BP.

GALECTIN-10 – THE MYSTERIOUS AUTO-CRYSTALIZING PROTEIN

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Galectin-10, also known as Charcot-Leyden Crystal (CLC) protein, auto-assembles into bipyramidal hexagonal crystals, hallmarks of eosinophil involvement in allergic inflammation. CLCs, found at sites of eosinophil infiltration in tissues, body fluids and secretions, were identified more than 150 years ago. CLC/Gal-10, a small 142-amino acid 16.5kDa protein was initially identified as eosinophil lysophospholipase, but was since assigned to the galectin superfamily based on amino acid sequence, three-dimensional structure, and genomic organization. We showed CLC/Gal-10 lacks intrinsic lysophospholipase activity, its weak enzymatic activity being due to contamination by a highly active 75kDa 'pancreatic' enzyme expressed by eosinophils.

The CLC/Gal-10 crystal structure identifies a carbohydrate recognition domain (CRD) with similarities to and key differences from galectins; CLC/Gal-10 does not bind β-galactosides, but binds mannose ***en crystal*** in an unusual conformation different from binding of lactosamines by related galectins; the significance of mannose binding remains equivocal. Partial conservation of residues involved in glycan binding leads to significant changes in topology and chemical nature of the CRD, with implications for glycan recognition ***in vivo***, providing clues toward identifying relevant ligands for CLC/Gal-10 and its role in eosinophil biology.

Southwestern (ligand) blotting, co-purification, co-immunoprecipitation and confocal microscopy show CLC/Gal-10 interacts ***in vitro*** and intracellularly in activated eosinophils with the glycosylated eosinophil cationic ribonucleases EDN and ECP, but not by glycan binding, and IFNγ induces rapid co-localization of CLC/Gal-10 with EDN and CD63. Because CLC/Gal-10 does not inhibit EDN ribonuclease activity, it likely functions as a carrier for vesicular transport of these ribonucleases during granulogenesis and piecemeal degranulation, enabling their extracellular functions without intracellular damage to the eosinophil. Notably, lentiviral shRNA knockdown of CLC/Gal-10 expression during eosinophil differentiation from CD34+ stem/progenitor cells induces a considerable defect in granulogenesis, implicating CLC/Gal-10 in this process.

Human regulatory T cells express CLC/Gal-10. Global proteomics of CD4+CD25+ Tregs identified CLC/Gal-10 as a novel biomarker, shown by siRNA knockdown to be essential for maintaining Treg anergy and T cell suppressive functions, but the mechanism by which it maintains CD4+CD25+ Treg phenotype is not established.

CLC/Gal-10 is a biomarker for eosinophil involvement and disease status in asthma, allergic rhinitis, eosinophilic esophagitis (EoE) and other diseases. We measured elevated CLC/Gal-10 levels by ELISA as a biomarker of active eosinophilic inflammation that is highly correlated with the numbers of esophageal eosinophils in EoE, and it's a potentially useful biomarker in induced sputum for identifying the eosinophilic asthma phenotype for guiding treatment. In celiac disease, CLC/Gal-10 expression was related to disease activity and eosinophil numbers in intestinal lesions, suggesting it a novel biomarker for evaluating eosinophil involvement in gluten intolerance. CLC/Gal-10 mRNA was reported to be a biomarker in whole blood for CRTH2 activation and inhibition by antagonists thereof. Lastly, genetic variations (SNPs) in the *CLC* promoter are potential susceptibility biomarkers for allergic rhinitis, variation compatible with recessive inheritance and increased CLC/Gal-10 in nasal fluid of patients with allergic rhinitis during allergy season.

Although the role of CLC/Gal-10 auto-crystallization remains a mystery, its intracellular functions in developing and activated eosinophils are becoming more 'crystal' clear.

Grant Support: Work on CLC/Gal-10 in the Ackerman and Acharya laboratories was supported by grants from the NIH/NIAID (AI25230, AI33043, SJA), MRC and Wellcome Trust (KRA), NIH T32 training grants DK007739 (CBD) and HL082547 (BTM), and the AAAAI T. Franklin Williams Scholar and UIC CCTS KL2RR029878 programs (SMN).

EOSINOPHIL SIGLECS AND THEIR LIGANDS

Bruce S. Bochner, MD

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Siglecs (sialic acid-binding immunoglobulin-like lectins) are I-type lectins found on the surface of most leukocytes. Among them, Siglec-8 on human eosinophils and its closest counterpart on mouse eosinophils, Siglec-F, are particularly prominent and selective in expression. To date, studies show that their engagement with antibodies or artificial polymeric sialoside ligands induces eosinophil apoptosis, especially in the context of cytokine priming that paradoxically would be expected to enhance survival. This makes Siglec-8 a promising therapeutic target to treat diseases involving eosinophils. This talk will compare and contrast known biology and signaling properties associated with Siglec-8 and Siglec-F in eosinophils. Similarities and differences between Siglec-8

and Siglec-F α2,3-linked sialoside ligands and endogenous tissue ligands (e.g., from the airways) will also be discussed, along with ongoing efforts to leverage another aspect of Siglec biology, namely its internalization following ligand engagement, as a way to preferentially deliver drugs including toxic payloads into Siglec-8-expressing cells. Finally, given the fact that Siglec-8 is only expressed in humans and some great ape species, preclinical testing of Siglec-8 targeting has been limited. The successful development of a new mouse strain molecularly engineered to express Siglec-8 only on eosinophils will also be presented.

Grant support: This work was supported by the National Heart, Lung, and Blood Institute (P01HL107151 and the National Institute of Allergy and Infectious Diseases (AI072265).

Disclosure: Dr. Bochner is on the scientific advisory board for Allakos Inc.; has several patents related to Siglec-8 that belong to The Johns Hopkins University School of Medicine, some of which have been licensed to Allakos; is entitled to receive a share of royalties from Allakos regarding milestones and the sale of Siglec- 8-related products; and owns stock in Allakos.

EOSINOPHILS, GALECTINS AND GLYCANS

Savita P. Rao, Xiao Na Ge and P. Sriramarao

Airway allergic inflammation including asthma is characterized by the presence of large numbers of eosinophils in the airways. Eosinophils can rapidly release inflammatory mediators such as cytokines, chemokines, growth factors and cytotoxic granule proteins upon stimulation, and play a critical role in the pathogenesis of allergic asthma. Identifying key players and understanding the molecular mechanisms that regulate eosinophil trafficking and recruitment to inflamed airways is a key to developing therapeutic strategies to limit their influx and ameliorating allergic asthma. Recent studies have brought to light the important role of glycans and glycan binding proteins in the recruitment of eosinophils. In addition to the role of previously identified eosinophil- and endothelial-expressed adhesion molecules in mediating eosinophil trafficking and recruitment to the inflamed airways, studies have also indicated a role for galectins in this process. Galectins are mammalian lectins expressed by various cell types including eosinophils that interact with β-galactosides on cell surface-expressed glycans to regulate cellular responses like production of inflammatory mediators, cell adhesion, migration and apoptosis. Depending on the type (galectin-1 or galectin-3) and location (extracellular or intracellular, endogenous or exogenously delivered), our studies have shown that galectins can differentially regulate eosinophil recruitment, activation and apoptosis and exert either pro- or anti-inflammatory outcomes. The physiologic role of glycan and galectins in the regulation of eosinophil recruitment and pathogenesis of allergic asthma will be discussed.

LABORATORY TECHNIQUES AND RESOURCES FOR MOUSE EOSINOPHILS

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Polymorphonuclear cells that stain red with eosin can be found in most Chordata suggesting a significant evolutionary role for these cells in a variety of organisms. In mouse and human the difference between eosinophil cytological appearances is quite evident upon hematoxylin eosin staining. Human eosinophils are bi-lobed with large red granules while mouse eosinophils vary in their nuclear morphology from rings, figure eights, to complex structures with often fainter staining of the granules with eosin. Moreover the stimulating agents that induce degranulation of these cells can vary significantly, with human eosinophils tending to have a wider array of inducers and mouse eosinophil stimulants remaining less defined. Yet, the functions of eosinophils in humans in both homeostasis and disease are largely unknown. In part this is due to the inability to easily manipulate human eosinophils to ask reductionist questions regarding their roles at baseline and in disease. For example, it would be quite problematic and difficult to remove a specific eosinophil gene and then return the cell to the patient to ask its function. Despite these mouse and human variances, mouse eosinophils provide a unique tool to understand the role of eosinophils in an organism's health and disease. Mouse eosinophils retain greater than 90% of the same genome as human eosinophils, are found in the same physiological locations in homeostasis and in disease states, and express many similar molecules. As such, the ability to isolate eosinophils from genetically modified mice, disease modeled mice, or from homeostatic conditions and perform 'omic' assessments or culture under specific conditions or transfer into a new novel animal has provided the ability to ask and answer unique specific questions about the roles of eosinophils in disease and health. This talk will highlight the several techniques that have been developed by laboratories over the last few decades to isolate pure eosinophils. These techniques include magnetic bead selection, bone marrow-derived culture, and flow cytometry sorting. Once isolated these eosinophils may also be manipulated prior to adoptive transfer into mice (e.g. eosinophil-deficient mice). The pros and cons, the technical considerations, as well as novel mouse models and tools available to complete reductionist studies of eosinophil functions will be discussed. To date these techniques and models have provided a plethora of knowledge demonstrating that eosinophils are necessary for both homeostatic activities as well as specific immunological functions on other cells during disease processes.

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Invited Speaker Abstracts

WHAT I NEED TO KNOW ABOUT EOSINOPHILS AND TRANSCRIPTION FACTORS

Patricia Fulkerson

Dynamic gene expression is a major regulatory mechanism that directs hematopoietic cell fate and differentiation, including eosinophil-lineage commitment and eosinophil differentiation. Recent advances in molecular experimental tools using purified eosinophil developmental stages and genetically-altered mice have led to identifying new regulators of eosinophil development. Understanding how specific signals direct distinct patterns of gene expression required for the specialized structures and functions of eosinophils will likely lead to new targets for therapeutic intervention.

PIN1 NULL EOSINOPHILS SHOW IMPAIRED DIFFERENTIATION AND SURVIVAL AFTER TLR7 SIGNALING

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Background: The response of eosinophils to respiratory virus infection has emerged as an important link between infectious pathogens and allergic exacerbations. Eosinophils contribute to antiviral host defense in the lung after activation of toll-like receptor (TLR) signaling. The *cis-trans* prolyl isomerase, Pin1 has been shown to modulate host immune responses to viral and allergen challenges.

Methods: Examined the effect of selective Pin1 deletion on eosinophilopoiesis and allergic airway inflammation in the context of ssRNA viral infection.

Results: Pin1 null eosinophils ($Pin1^{fl/fl}$: eoCre) (driven by EPX promoter) were differentiated in vitro from bone marrow with mature cells normal in maturity, yield and viability as compared to WT eosinophils ($Pin1^{fl/fl}$). Incubation of WT or Pin1 null, mature eosinophils (D14) with TLR7 agonists (R848 or ssRNA) showed similar IL5-mediated cell survival. However, when TLR7 agonists were added to immature cells (D8), the viability of KO eosinophils were reduced by nearly 90% after 8 days (D16) compared to WT cells. Notably, these effects were observed exclusively with TLR7 agonists. Subsequent analysis revealed that, unlike WT cells, immature eosinophils lacking Pin1 were unable to fully activate the ER stress-induced unfolded-protein response (UPR) pathways. KO cells showed significantly reduced expression of UPR components (Xbp1, ATF-4 and ATF-6α), lack of Xbp1 mRNA splicing and ATF-6 protein cleavage, and reduced expression of downstream target genes. Consistent with these in vitro observation, intratracheal (IT) or IV instillation of TLR-7 agonists (R848 or rhinovirus) into KO mice with allergic asthma resulted in significantly lower eosinophil numbers in BAL, blood and bone marrow and reduced EDN and IFN-α/β production in the lung compared to WT controls ($Pin1^{fl/fl}$).

Conclusions: These data strongly suggest that Pin1 is required for bone marrow eosinophil differentiation in the presence of TLR7 activation (e.g. syncytial, pneumonia and rhinovirus), type 1 interferon responses and pulmonary eosinophil migration during concurrent allergen challenge and viral infection.

PHENOTYPIC AND FUNCTIONAL CHANGES IN EOSINOPHILS MAY PROMOTE ANTIVIRAL RESPONSES IN HOSTS WITH ALLERGIC ASTHMA

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Background: Respiratory virus infections have been associated with an increased incidence of asthma exacerbations. However, the impact such virus-induced exacerbations have on viral pathogenesis in the allergic host is not clear. Epidemiologic data surrounding the 2009 influenza pandemic identified that asthmatics were more likely to be hospitalized but less likely to suffer from severe influenza compared to non-asthmatics. Our animal models also showed that allergic inflammation in the airways at the time of virus infection protects the host from influenza-mediated pulmonary cytopathology and disease. Therefore, we hypothesized that eosinophils in the lungs dynamically respond to influenza A virus (IAV) to help mitigate influenza pathogenesis by boosting cellular immunity.

Methods: Phenotypic and physiologic responses in eosinophils that were exposed to IAV were determined by transmission electron microscopy, flow cytometry, and Seahorse metabolic analyzer. Eosinophil activation was measured in various niches (including bone marrow, thymus, lymph nodes, and lungs) by flow cytometry. Adoptive transfer of eosinophils into mice infected with IAV was performed to determine changes in influenza disease pathogenesis. Mice infected with genetically modified IAV viruses were used to determine the ability of eosinophils to activate cellular immune responses against IAV. *In vitro* assays were performed to determine the impact of virus-exposed eosinophils on $CD8^+$ T cell responses.

Results: Activation markers and eosinophil trafficking in allergen-challenged mice differed during IAV infection. Eosinophils were susceptible to IAV infection, underwent piecemeal degranulation, increased MHCI expression (among other markers), and became activated in response to IAV. Oxygen consumption and extracellular acidification rates were unaffected in IAV infected eosinophils during early infection. Virus infected mice that received eosinophils had reduced viral burden, improved lung compliance, and increased $CD8^+$ T cell recruitment. Antigen-specific activation of $CD8^+$ T cells was induced by eosinophils wherein $CD8^+$ T cells formed

immune synapses, proliferated, and produced antiviral cytokines in response.

Conclusions: Dynamic eosinophil responses to influenza virus during allergic asthma may enhance cellular immune responses to promote viral clearance and mitigate influenza disease pathogenesis.

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A NOVEL BIASED ANTAGONIST OF THE EOTAXIN-CCR3 PATHWAY IN EOSINOPHILS

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Background: CCR3 is a highly promiscuous GPCR, interacting with several inflammatory chemokines, including high affinity agonists: eotaxin (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), and RANTES (CCL5). CCR3 and its principal ligands play a prominent role in the pathogenesis of allergic diseases, including asthma and EoE, and this receptor has long been a target of drug discovery. Several small molecule antagonists have been developed to date; however, these inhibitors failed to enter phase II/III clinical trials or lacked efficacy. A possible underlying cause of this failure is these unbiased antagonists' mode of inhibition that prevents receptor internalization and degradation, leading to drug tolerance. To address this, we sought to develop a novel nanoparticle peptide-based CCR3 antagonist (R321) with a biased mode of inhibition.

Methods: Self-assembly of the inhibitory R321 peptide into nanoparticles was analyzed by Dynamic Light Scattering (DLS). The inhibitory effect on CCR3 signaling was assessed by determining IC_{50} and IC_{90} values for eotaxin-induced CCR3-mediated chemotaxis of peripheral blood eosinophils and of a CCR3+ eosinophil cell line, AML14.3D10-CCR3. Mechanism of inhibition was determined by assessing ERK 1/2 phosphorylation in CCR3+ cells by means of western blotting. Colocalization of the CCR3 with β-arrestin 2 was assessed by confocal microscopy and analyzed using Pearson's method. Binding of R321 to reductively methylated CCR3 membrane preparations in the absence and presence of CCL11 was analyzed by NMR.

Results: The R321 peptide, derived from the second transmembrane helix of CCR3, self-assembles into monodisperse nanoparticles (radius of 7.1 ± 0.7 nm, 7.2% polydispersity). IC_{50}/IC_{90} values for eotaxin induced CCR3-mediated chemotaxis of blood eosinophils were: 0.11± 0.01 μM/ 0.76± 0.10 μM (CCL11); 0.08 ± 0.01 μM/ 0.73 ± 0.10 μM (CCL24); and 0.16 ± 0.02 μM/ 1.10 ± 0.15 μM (CCL26). Unlike two other tested small molecule inhibitors, R321 inhibited only the early phase of ERK1/2 activation (5 min post-stimulation) and not late phase activation (30 min post-stimulation) associated with β-arrestin recruitment, receptor internalization and degradation. β-arrestin recruitment to CCR3 upon inhibition with R321, but not with small molecule inhibitors, was confirmed by colocalization in confocal microscopy images and Pearson's colocalization coefficients. R321 alone or with CCL11 ligand promotes CCR3 endocytosis and degradation, and is cell- and CCR3-specific, selectively inhibiting eosinophil chemotaxis to CCR3 ligands but not to CXCL12/CXCR4-induced chemotaxis of Jurkat T cells. NMR spectra of reductively methylated CCR3 enriched membranes showed binding of R321 to CCR3 in the presence of CCL11. Even 50 μM R321 did not displace 1 μM CCL11, indicating a non-competitive allosteric mode of inhibition.

Conclusions: We evaluated the activity of a novel CCR3 self-assembling nanoparticle peptide antagonist with a potent inhibitory effect on CCR3-mediated signaling and chemotaxis of a CCR3+ eosinophil cell line and primary blood eosinophils. By demonstrating a selective inhibition of only the desired subset of the CCR3 signaling cascade, this novel biased antagonist may hold significant therapeutic promise by preventing receptor accumulation on the cell surface, thus eluding the development of drug tolerance, an issue for biased antagonists of GPCRs in general.

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AIOLOS REGULATES EOSINOPHIL MIGRATION

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Background: Accumulation of an abnormally high number of eosinophils (Eos) in tissues often results in damage and organ dysfunction in allergic disease. Preclinical and clinical studies have established that CCR3 ligands (CCL11, CCL24, CCL26) are critical for disease-associated tissue eosinophilia. Yet, blockade of the individual mediators (e.g. CCR3, CCL11) has been insufficient to completely resolve tissue eosinophilia, likely due to simultaneous local expression of multiple Eos-attracting mediators acting in parallel. Thus, delineating the molecular mechanisms that control Eos trafficking into tissues is clinically significant. With the goal to identify

new regulators of Eos development and functional responses, we previously reported results from transcriptional profiling of murine eosinophil progenitors (EoPs) and eosinophils, including expression of the transcription factor Aiolos in murine EoPs as well as in mature Eos in mice and humans. Notably, Aiolos binding sites were enriched in the promoters of genes expressed by EoPs and Eos, including genes involved in chemotaxis. We investigated the role of Aiolos in eosinophil migration.

Methods: To evaluate the effect of the loss of Aiolos on eosinophil function, we performed chemotaxis and functional assays with WT and Aiolos-deficient (Aiolos KO) LDBM-derived eosinophils. To determine the role of Aiolos in eosinophil migration in allergic inflammation, we tested adoptive transfer models of bone marrow-derived eosinophil or antigen-specific CD4+ Th2 cell. To be able to distinguish between donor and recipient cells, we used congenic mice with CD45.1 vs CD45.2 markers. The presence of eosinophils in the BALF were determined by flow cytometry staining.

Results: The migratory response of AiolosKO Eos is markedly impaired toward CCR3 ligands in vitro and in vivo, along with significantly reduced mRNA and surface expression of CCR3. Signaling pathway as well as actin polymerization assay show a decrease in activation in AiolosKO eosinophils by a CCR3 ligand. We also demonstrate a profound reduction in Eos accumulation in the small intestine of AiolosKO mice. These latter findings provide in vivo relevance to our findings as intestinal eosinophilia is CCL11 and CCR3 dependent. We further show that migration of adoptively transferred AiolosKO Eos into the airway of allergen-challenged mice was significantly reduced when compared to WT Eos in an experimental model of asthma. Moreover, airway inflammation driven by adoptively transferred antigen-specific CD4+Th2 cells into WT or AiolosKO mice demonstrate a decrease of eosinophil recruitment to the lungs, highlighting an intrinsic defect in tissue trafficking in the AiolosKO Eos.

Conclusion: Collectively, our studies implicate Aiolos as a global regulator for eosinophil migration.

A PHENOTYPICALLY DISTINCT SUBSET OF EOSINOPHILS IS RECOVERED WITH INTESTINAL INTRAEPITHELIAL LEUKOCYTES

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Background: Eosinophils naturally home to most regions of the gastrointestinal tract. Several allergic and inflammatory diseases are associated with increased numbers of intestinal eosinophils, although the role(s) of eosinophils in the initiation and pathogenesis of intestinal inflammatory diseases remains enigmatic. We recently demonstrated that resident intestinal eosinophils acquire antigen from the intestinal lumen in allergensensitized (but not naïve) mice (Smith et al. *JI* 2016; 197:3716). In this study we extend these findings by investigating the phenotype and localization of resident intestinal eosinophils at baseline, within the context of systemic allergic sensitization and following remote (pulmonary) allergen challenge.

Methods: Intestinal lamina propria (LP) and intraepithelial (IE) leukocyte populations were isolated following standard protocols and purity confirmed by assessing appropriate lymphocyte markers. Intestinal eosinophils were identified in leukocyte preparations isolated from naïve BALB/c and C57BL/6 wild type mice as liveCD45+SSChiSiglecFhi cells. Eosinophil identity of the gated population was confirmed by CCR3 expression, the absence of this population in eosinophil deficient mice, and by morphological and tinctorial confirmation of sorted cells. Localization of eosinophils to LP and IE compartments was further confirmed by identification of GFP+ eosinophils within LP and IE preparations of EoCre+/-mTmGfl/fl mice by flow cytometry. (Eosinophil targeted Cre-recombinase expressing mice (EoCre+/-) were provided by Drs. Nancy and Jamie Lee, Mayo Clinic Arizona). Surface phenotypes of eosinophils were assessed by flow cytometry. Endotracheal administration of allergen was used to determine the effect of remote allergen challenge on intestinal eosinophils in allergen-sensitized mice. For assessment of in vivo uptake of intestinal lumen-derived antigen in live mice, fluorescently-labeled ovalbumin or bovine serum albumin was injected into the intestinal lumen of anesthetized mice using an intestinal loop surgical model.

Results: Eosinophils recovered from LP preparations were distinguished from blood eosinophils by increased surface expressions of CD11b and CD11c and constitutively expressed moderate levels of molecules associated with antigen presentation (i.e. MHC II and CD80). Unexpectedly, eosinophils were also routinely detected within IE preparations from naïve and allergen-challenged mice. IE eosinophils were phenotypically distinguished from LP eosinophils by increased expressions of Siglec F, CD11b and CD11c with low to no detectable surface MHC II. Remote pulmonary exposure of sensitized mice to allergen increased the total number and frequency of both LP and IE eosinophil populations within the antigen unexposed small intestine, and both LP and IE eosinophil populations from allergen-sensitized mice acquired fluorescently-labeled soluble antigens deposited directly into the intestinal lumen within 45 minutes of allergen delivery.

Conclusions: These findings identify a novel subset of tissue-dwelling eosinophils present at baseline within the intestinal IE niche, provide new phenotypic insights into the preparedness of resident intestinal eosinophils to engage in antigen-specific functions,

and reveal a link between remote mucosal allergen exposure and the frequency of intestinal eosinophils that may shed light on the relationship between aeroallergens and eosinophilic gastrointestinal inflammation.

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WHOLE EXOME RESEQUENCING IDENTIFIES PUTATIVELY FUNCTIONAL RARE VARIANTS ASSOCIATED WITH EOSINOPHILIC GASTROENTERITIS

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Background: One presentation of hypereosinophilic syndrome (HES) is eosinophilic gastroenteritis in which patients suffer from recurrent episodes of griping abdominal pain, bloating, nausea and vomiting and changed bowel habit. Ascites can occur, but is unusual. The small bowel and colonic mucosa is generally histologically normal with no evidence of colitis, although small bowel thickening and oedema may be noted during severe exacerbations on CT scan and the faecal calprotectin may be modestly raised. The condition is variable in severity and may be associated with only a modest eosinophilia below the HES threshold. It is responsive to oral corticosteroids. The aetiology is not known. We have identified a small family with this condition. The index cases were a brother and sister who both suffer from a severe form of the disease. The brother also has severe eosinophilic asthma. Subsequently the mother was found to have a lifelong history of similar although milder symptoms associated with an eosinophilia for which she had never sought medical attention. We then identified the daughter of one of the index cases with typical symptoms and an eosinophilia who similarly had never sought medical attention.

Methods: We undertook whole-exome resequencing on three of the family members (index cases and the mother). Three unaffected unrelated controls from the same clinic were re-sequenced in the same experiment. Genetic data from the subjects was also compared with multiple databases of genetic variation.

Results: Putatively functional variants that were unique to the three cases were identified. Seven functional missense variants in seven genes were seen in all three cases and in none of the resequenced controls or the databases of genetic variation. The genes were identified as *NCKAP5, PLXNB1, MYLIP, POC1B-GALNT4*, *MTA1, CMTM3* and *PLCG1*.
None of the genes were identified as strong candidate genes based on a literature search. Following bioinformatic analysis of gene ontology and protein expression databases, *PLCG1* (Phospholipase C, gamma 1) and *CMTM3* (CKLF-like MARVEL transmembrane domain containing 3) were found to have the most evidence linking them to the hypereosinophilia observed in the family. *CMTM3* belongs to the chemokine-like factor gene superfamily, suggesting a link with hypereosinophilia via the role of chemokines in the migration of eosinophils, while *PLCG1* plays a role in the intracellular transduction of receptor-mediated tyrosine kinase activators. *CMTM3* and *PLCG1* had medium/high levels of protein expression in the stomach, duodenum, small intestine, colon and lungs. *PLCG1* also had medium/high levels in the oesophagus, lymph node and bronchus.

Conclusion: Further work is needed to prioritise the list of variants through recruitment of additional affected and unaffected family members. Identification of the mutation responsible for what appears to be an autosomal dominant Mendelian expression of HES may offer insights into the causes of eosinophilic gastroenteritis in particular and HES in general.

TRIB1 REGULATES EOSINOPHIL IDENTITY BY RESTRAINING THE NEUTROPHIL IDENTITY PROGRAM

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Background: Granulocytes, including neutrophils and eosinophils, are critically important for host defense and homeostasis, yet can also induce immunopathology. At present, there are significant gaps in understanding how granulocytes differentiate from bone marrow (BM) progenitors into mature effectors. The pseudokinase tribbles homologue 1 (Trib1) influences neutrophil and eosinophil development and modulates levels of the transcription factor C/EBPα. How and when Trib1 functions in eosinophil and neutrophil identity is not known. Here we evaluated the influence of Trib1 on eosinophil development and identity.

Methods: We generated mice lacking Trib1 in all hematopoietic cells using VavCre and specifically in eosinophil-lineage cells using eoCre. We used both in vivo and ex vivo methods to study granulopoiesis in cells from these mice. We examined in vivo mouse granulopoiesis using flow cytometry and IL-5 cultures to generate eosinophils ex vivo from BM. C/EBPα knockdown was done using shRNA.

Results: We confirmed that the absence of Trib1 in hematopoietic cells expands neutrophils and decreases peripheral eosinophils. To determine how Trib1 acts in eosinophil development, we found that Trib1 expression increased during differentiation, beginning in eosinophil lineage-committed progenitors (EoP). Furthermore, deleting Trib1 beginning in the HSC using VavCre selectively decreased EoP, while preserving the common myeloid and granulocyte/macrophage progenitors (CMP/GMP). In contrast, deleting Trib1 with eoCre after eosinophil lineage commitment resulted in minimal change in EoP frequency. Notably, regardless of deletion timing, Trib1-deficient BM developed a population of atypical granulocytes in the BM with eosinophilic and neutrophilic characteristics, indicating abnormal fate regulation. In vivo eosinophil lineage tracing revealed that these atypical granulocytes derived from EoPs and did not transition to other lineages. In the absence of Trib1, ex vivo eosinophil production was markedly reduced, despite the presence of IL-5. In contrast, Trib1-deficient BM readily generated neutrophils in eosinophil-differentiating conditions. Mechanistically, we found that Trib1-deficient granulocytes expressed increased amounts of C/EBPα p42, and knockdown of Cebpa increased eosinophil output in IL-5-cultured Trib1-deficient BM.

Conclusions: Using temporally controlled deletion, we identified roles for Trib1 in modulating multiple aspects of development, from a requirement in eosinophil lineage commitment to maintenance of cell identity during terminal differentiation. These data indicate that Trib1 regulates cell fate partly by tuning levels of C/EBPα, as increased C/EBPα lead to cells with both neutrophilic and eosinophilic features. This provides insight into how precise C/EBPα levels balance granulocyte identity. Our data suggest that Trib1 actively represses the neutrophil gene program in developing eosinophils via regulating C/EBPα. Our data also raise the possibility that Trib1 itself does not induce and/or maintain the eosinophil program, as cells with both eosinophil and neutrophil characteristics are present in the absence of Trib1. Finally, our work defines the function of Trib1 in eosinophil development and begins to identify myeloid factors that restrain alternative lineage identity among granulocytes.

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EXOSOMES FROM EOSINOPHILS AUTOREGULATE AND PROMOTE EOSINOPHIL FUNCTIONS

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Backgraund: Eosinophils are able to secrete exosomes that play an undefined role in asthma pathogenesis. We hypothesized that exosomes released by eosinophils autoregulate and promote eosinophil function.

Methods: Eosinophils of asthmatic patients (*n*=58) and healthy volunteers (*n*=16) were purified from peripheral blood and exosomes were isolated and quantified from eosinophils of asthmatic and healthy population. Apoptosis, adhesion, adhesion molecules expression and migration assays were performed with eosinophils in the presence or absence of exosomes from healthy and asthmatic individuals. Reactive oxygen species (ROS) were evaluated by flow cytometry using intracellular fluorescent probe and nitric oxide (NO) using colorimetric kit. Also, exosomal proteins were analyzed by mass spectrometry.

Results: Eosinophil-derived exosomes induce an increase in NO and ROS production on eosinophils. Moreover, exosomes can act as a chemotactic factor on eosinophils, and they produce an increase in cell adhesion giving rise to specific augment of adhesion molecules such as ICAM-1 and integrin α_2. Protein content between exosomes from healthy and asthmatic individuals seems to be similar in both groups.

Conclusion: In conclusion, we describe that exosomes from eosinophils of asthmatic patients can modify several specific eosinophil functions related to asthma pathogenesis and they could contribute to development and maintenance of asthma in a fundamental way.

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VACUOLATED EOSINOPHILS DRIVE MALADAPTIVE IMMUNITY IN EXPERIMENTAL ALLERGIC ASTHMA THROUGH THE C5a-C5aR1 AXIS

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Background: Recently, we identified a previously unrecognized vacuolated eosinophilic population (vEOS), expressing CD11c and

C5aR1 in the course of experimental allergic asthma. Vacuolated eosinophils, present in the blood, were already reported as biomarkers for asthma-exacerbations in children. Here we aimed to define the importance of vEOS for the development of experimental allergic asthma.

Methods: We used ovalbumin and house-dust-mite (HDM)-mediated allergic asthma models with C57BL/6 wt and GFP-C5aR1 knock-in or BALB/c wt and C5aR1-/- mice. Additionally, we used a model of IL-33-mediated airway hyperreactivity and an adoptive transfer model using house dust mite-pulsed pulmonary vEOS or conventional dendritic cells (cDCs). Lung residential eosinophils (EOS), vEOS, and T cells were identified and characterized in bronchoalveolar lavage fluid (BAL), lung, and mediastinal lymph-node (mLN) cell isolations by flow cytometry, Giemsa staining, immunofluorescence and electron microscopy. Data are representative of at least two independent experiments and statistical analysis was done by a one-way ANOVA or Student's t-test.

Results: In both models, we found EOS in the airways and the lung. In contrast, vEOS ocurred solely in the lung and the mLN under asthmatic conditions. EOS and vEOS expressed similar levels of CCR3, ST2 and F4/80 and showed signs of piecemeal degranulation in electron-microscopy. Pulmonary vEOS were located near arterys and airways, but not in the alveolar compartment, and were in close contact with T cells. Furthermore, pulmonary vEOS but not EOS took up, processed, and accumulated antigen, expressed MHC II and the co-stimulatory molecules CD80 and CD86. In mLN, vEOS upregulated MHC II, CD40, and CD86. In established allergic asthma, we found upregulation of C5aR1 in pulmonary EOS and vEOS using the GFP-C5aR1 reporter mouse, which was higher in vEOS than in EOS. Also, we found high intracellular C5 and C5aR1 expression in vEOS but not in EOS suggesting non-canonical C5a generation and intracellular C5aR1 activation in vEOS. Finally, the number of vEOS in the mLN was significantly reduced and the upregulation of CD40 and CD86 was abrogated in C5aR1-/- as compared with wt mice.

In co-culture with OVA-transgenic T cells (DO11.10 RAG2-/-), pulmonary vEOS and cDCs drove strong T cell proliferation. Of note, vEOS/T cell co-cultures induced significantly more divisions than cDC/T cell co-cultures. cDC/T and vEOS/ T cell co-cultures resulted in robust IL-13 and IL-17 production. In addition, we found also high IL-10 in cDC/T cell co-cultures Surprisingly co-culture of vEOS, cDCs and T cells showed a significant increase in IL-17 production. Adoptive transfer of ex vivo HDM-pulsed vEOS or cDCs both induced a robust Th2 (IL-13) but no Th17 response.

Conclusions: Collectively, our data demonstrate that vEOS exert strong antigen-presenting properties, which are controlled by the C5a-C5aR1 axis. Further, they activate T cells in vitro and in vivo, where they contribute to TH2 and TH17 responses in vitro and a TH2 response in vivo. Thus, vEOS act in concert with cDCs to drive maladaptive immunity in experimental allergic asthma.

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WHEN IS A WILD TYPE MOUSE NOT WILD TYPE?

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Background: Wild-type littermates are commonly used as controls for transgenic animals. However, environmental exposures during fetal development affect disease manifestations later in life, independent of genotype. Since asthma studies frequently utilize interleukin-5 (IL5) transgenic mice to determine the role of eosinophils in airway pathology, we tested whether overexpression of IL5 in maternal mice or fetal littermates subsequently affects airway physiology in genetically wild-type (WT) mice.

Methods: NJ1726 mice have an IL5 transgene driven by an airway epithelial promoter (i.e., CC10). WT mice used for breeding were purchased from Jackson Laboratories. The following combination of breeding pairs were established (all mice are on a C57BL/6J background):

Female (F)		Male (M)
WT	x	WT
WT	X	NJ1726 hemizygote
NJ1726 hemizygote	x	NJ1726 hemizygote

NJ1726 hemizygote and genotypically WT offspring from breeding pairs were used. Mice were sedated, intubated, and mechanically ventilated. Changes in airway resistance in response to inhaled serotonin were recorded. Bronchoalveolar lavage (BAL) IL5 and substance P levels were measured with ELISA.

Results: WT offspring from an NJ.1726/+ (♀) x NJ.1726/+ (♂) cross had significantly increased bronchoconstriction in response to serotonin compared to WT offspring from a WT(♀) x WT (♂) cross. Increased bronchoconstriction occurred with maternal or fetal littermate transgene expression. Paternal transgene expression increased bronchoconstriction only when fetal littermates expressed IL5 (offspring from WT(♀) x NJ1726/+ (♂)). WT offspring had low BAL eosinophils (WT offspring from NJ.1726/+ (♀) x NJ.1726/+ (♂), 0.2±0.3%, n=6; WT offspring from WT(♀) x WT (♂) crosses had no evidence of airway eosinophjils, 0±0%, n=6) compared with NJ1726/+ offspring (23±7%, n=6). WT offspring had non-detectable IL5 in BAL as compared with NJ.1726 mice. WT offspring from

NJ1726/+ (♀) x NJ.1726/+ (♂) parents had elevated BAL substance P (2361±894 pg/mL) compared with WT offspring from WT(♀) x WT (♂) crosses (1035±768, p=0.0203).

Conclusions: Exposure to IL-5 and/or eosinophils in utero alters baseline airway physiology and substance P levels in WT offspring. Our data call into question the practice of using WT littermates as controls for transgenic animals. At the same time, these data suggest IL5tg mice may serve as a model for studying the effects of maternal asthma on fetal airway development.

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MEPOLIZUMAB FOR THE TREATMENT OF PATIENTS WITH EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS: A PHASE III RANDOMIZED, PLACEBO-CONTROLLED TRIAL

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Background: Eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss) is a systemic vasculitis associated with asthma, eosinophilia, sinusitis, pulmonary infiltrates, and neuropathy. In other hypereosinophilic syndromes and eosinophilic asthma, the anti-interleukin-5 monoclonal antibody mepolizumab has been shown to reduce blood eosinophil counts with concomitant clinical improvement.

Methods: We conducted a Phase III, randomized, placebo-controlled, double-blind, parallel-group, multi-center study (NCT02020889) in patients with EGPA and a history of relapsing or refractory disease on stable therapy with prednisolone/prednisone ≥7.5–≤50mg/day with or without additional immunosuppressive therapy for ≥4 weeks. Patients were randomized 1:1 to receive mepolizumab 300mg or placebo subcutaneously, in addition to standard of care, every 4 weeks for 52 weeks. After Week 4, glucocorticoid dose could be tapered, per physician judgment, according to a suggested standard-of-care protocol. Co-primary endpoints, based on an intent-to-treat analysis, were accrued duration of remission (Birmingham Vasculitis Activity Score [BVAS]=0, prednisolone/prednisone dose ≤4mg/day) over 52 weeks; and the proportion of patients in remission at both Weeks 36 and 48. Secondary endpoints included average glucocorticoid dose during Weeks 49–52 and time to first EGPA relapse. Safety was also assessed.

Results: The intent-to-treat population included 136 randomized patients (mepolizumab n=68, placebo n=68). Baseline characteristics were similar between groups. Duration of remission accrued over 52 weeks was significantly prolonged with mepolizumab vs placebo (odds ratio: 5.91 [95% confidence interval [CI]: 2.68, 13.03]; p<0.001); a significantly higher proportion of patients were in remission at Weeks 36 and 48 (32% vs 3%, respectively, odds ratio: 16.74 [95% CI: 3.61, 77.56]; p<0.001). Significant reductions in average daily glucocorticoid dose during Weeks 49–52 were seen with mepolizumab vs placebo (odds ratio: 0.20 [95% CI: 0.09, 0.41]; p<0.001). Median (range) prednisolone/prednisone dose during Weeks 49–52 was 5.0 (0.0–113.4) mg/day in the mepolizumab group and 10.0 (0.0–46.3) mg/day in the placebo group. Time to first EGPA relapse was significantly longer with mepolizumab vs placebo (hazard ratio: 0.32 [95% CI: 0.21, 0.50]; p<0.001). Rates of adverse events (AEs) and serious AEs were similar for mepolizumab and placebo.

Conclusions: Treatment with mepolizumab significantly increased the likelihood and duration of remission while reducing glucocorticoid use in patients with EGPA, with a safety profile consistent with previous studies in severe asthma and EGPA. This demonstrates consistent and meaningful clinical benefits of mepolizumab in patients with EGPA.

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SINGLE-CELL ANALYSIS OF HUMAN T CELLS IN EOSINOPHILIC INFLAMMATION

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Background: T cells orchestrate eosinophilic inflammation in allergic diseases, yet the bulk of data concerning these cells have been derived from analysis of peripheral blood–derived cells, which has limited sensitivity to capture their full effector functions in the tissue. Herein, we aimed to elucidate tissue-residing T cells at the single-cell level using single-cell RNA sequencing.

Methods: We developed a comprehensive platform based on analysis of tissue digests of esophageal biopsies obtained from patients with marked eosinophilic inflammation by performing RNA sequencing (RNA-seq) of pooled $CD3^+$ T cells and single-cell analysis by FACS RNA-seq. Analysis of control biopsies and $CD3^+$ T cells from autologous blood was also performed. The C1 Fluidigm system was used to capture FACS-sorted $CD3^+$ tissue lymphocytes via the 96-cell platform, followed by on-chip cDNA library generation. Next-generation sequencing (NGS) was performed by Illumina HiSeq 2500 with a pair-ended 75-bp read length and of 2 million reads per single T cell. NGS data alignment and analysis were performed by Strand NGS software.

Results: Herein, we demonstrate that in contrast to circulating $CD3^+$ T cells, tissue $CD3^+$ T lymphocytes have a disparate transcriptome and surface phenotype (assessed by FACS and bulk $CD3^+$-sorted RNA-seq) to accommodate several unique tissue effector functions. FACS analysis identified that CD25, CD40L, ST2, CCR3, CCR8, TSLP-R, CXCR4 and iCOS were relatively enriched on tissue-residing $CD4^+$ cells compared to tissue $CD8^+$ and blood $CD4^+/CD8^+$ T cells. Bulk $CD3^+$ RNA-seq revealed 343 genes differentially regulated between tissue and circulating $CD3^+$ T cells. Single-cell RNA-seq identified 16 classes of tissue $CD3^+$ T cells, as distinguished by 350 variation-contributing genes derived from principal component analysis. $CD3^+$ T cells present in eosinophilic inflammation were enriched in Th2 cytokine–producing Th cells and were characterized by unique expression of 30 genes as compared with other, less-pathogenic $CD3^+$ T cell populations. These 30 genes result in a unique phenotype of $CD45RO^+$ $CRTH2^+$ $HPGDS^+$ super-activated effector memory cells. Although cytokine production was limited to these pathogenic effector cells, individual cells expressed variable levels of IL-4, IL-5 and IL-13, demonstrating a heterogeneity within the pathogenic cluster.

Conclusions: We have elucidated the transcriptome of tissue-residing human $CD3^+$ T cells at the bulk and single-cell level by RNA-seq. In addition to providing a novel genetic resource to study these cells, we have uncovered the unique gene expression profiles of the Th2 cytokine–producing cells ($IL\text{-}5^+IL\text{-}13^+HPGDS^+CRTH2^+CD45RO^+$ memory effector Th cells) and their relationship with other $CD3^+$ T cells present in homeostatic and allergic eosinophilic human tissue.

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IL-33 DYSREGULATES REGULATORY T (Treg) CELLS AND IMPAIRS ESTABLISHED IMMUNOLOGICAL TOLERANCE IN THE LUNGS

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Background: Airway exposure to innocuous environmental antigens generally leads to immunological tolerance. A fundamental question remains: why is airway tolerance compromised in patients with allergic airway diseases, such as asthma, resulting in chronic eosinophilic airway inflammation? Interleukin (IL)-33 promotes innate and adaptive type 2 immunity and may provide the answer to this question.

Methods: The goal of this study was to investigate the roles IL-33 plays in altering regulatory T (Treg) cells in the lungs and in affecting previously established airway immunological tolerance. We analyzed $CD4^+$ forkhead box p3 $(Foxp3)^+$ Treg cells that were isolated from the lungs of naïve BALB/c mice and those treated with IL-33. Airway tolerance and allergen-induced eosinophilic airway inflammation models in mice were used to investigate how IL-33 affects established immunological tolerance *in vivo*.

Results: $CD4^+Foxp3^+$ Treg cells in the lungs expressed IL-33 receptor ST2. When exposed to IL-33, Treg cells upregulated their expression of canonical Th2 transcription factor GATA3 as well as ST2, and produced type 2 cytokines. Treg cells lost their ability to suppress effector T cells in the presence of IL-33. When mice were exposed intranasally to innocuous antigen, namely endotoxin-free ovalbumin (OVA), they developed airway immune tolerance to the antigen. When these mice were exposed intranasally to OVA in the presence of IL-33, they recovered the Th2-type immune response and developed eosinophilic airway inflammation when subsequently challenged with OVA. Furthermore, dysregulated $Foxp3^+$ Treg cells with distinct characteristics of Th2 cells increased in the lungs of the mice undergoing IL-33-dependent allergen-driven chronic eosinophilic airway inflammation.

Conclusions: IL-33 dysregulates lung Treg cells and impairs immunological tolerance to inhaled antigens. Established airway tolerance may not be sustained in the presence of an innate immunological stimulation, such as IL-33. IL-33 may play a key role in

chronic eosinophilic airway inflammation that is observed patients with allergic airway diseases.

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EOSINOPHILS ARE AN INTEGRAL PART OF THE TUMOR MICROENVIRONMENT IN COLORECTAL CANCER EXERTING POTENT ANTI-TUMORIGENIC ACTIVITIES

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Background: Tumor-associated eosinophilia has been first described more than 120 years ago, and is frequently observed in many types of cancer. Yet, fundamental knowledge is missing regarding the roles of tumor-associated eosinophils. Since gastrointestinal (GI) tract is the largest eosinophil reservoir in the body and a main organ for eosinophil-mediated diseases, we focused our studies on colorectal cancer (CRC) as a model system to define the roles of eosinophils in the tumor microenvironment (TME).

Methods: Eosinophil infiltration and function were studied in three independent models of colorectal cancer (CRC), representing the genetically driven and inflammation-driven CRC models (i.e. $Apc^{min/+}$ model and AOM+DSS treatment, respectively), as well as in colonic orthotopic injection of a tumor epithelial cell line.

Results: We report that eosinophils are an integral part of the TME in CRC. Eosinophils are actively and specifically recruited to the TME in the above mentioned models where they undergo degranulation. Consistently, analysis of "tumorigenic" and "uninvolved" biopsy sections of CRC patients revealed increased eosinophilic accumulation in the tumorigenic site. Moreover, we show that a single intravenous injection of eosinophils into eosinophil deficient mice (ΔdblGATA mice) undergoing CAC or ΔdblGATA/$Apc^{min/+}$ mice resulted in substantial recruitment of eosinophils to the TME, which fosters prolonged eosinophils survival likely via secretion of GM-CSF, IL-3 and IL-5.

Eosinophil-deficient ΔdblGATA mice undergoing CAC displayed significantly decreased survival, which was associated with increased tumor number and size. Similarly, generation of ΔdblGATA/$Apc^{min/+}$ mice revealed significantly decreased survival, which was associated with increased tumor burden, thereby suggesting an anti-tumorigenic role for eosinophils. Importantly, the anti-tumorigenic effects of eosinophils were independent of changes in $CD8^+$ T cells or MDSCs and were accompanied with a specific decrease in anti-active caspase 3^+ cells. No changes were observed in the numbers of Ki-67^+ or $CD31^+$ cells. Thus, supporting eosinophil-mediated cytotoxicity in-vivo. Indeed, eosinophils were capable of killing CRC cell lines in vitro.

Conclusions: Our data establish key anti-tumorigenic roles for eosinophils in CRC. These findings may facilitate the development of new pharmacological treatments unleashing robust anti-tumor responses by eosinophils and will hopefully lead to the development of innovative eosinophil-oriented "checkpoint inhibitors".

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EVIDENCE FOR A ROLE OF EOSINOPHILS IN BLISTER FORMATION IN BULLOUS PEMPHIGOID

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Background: Bullous pemphigoid (BP) is an autoimmune bullous disease of the skin characterized by subepidermal blister formation due to tissue-bound and circulating autoantibodies to the hemidesmosomal antigens BP180 and BP230. Although eosinophils and their toxic mediators are found abundantly in BP lesions, their role in blister formation has remained unclear. This study aimed at investigating the role of eosinophils in the pathogenesis of BP with a specific focus on blister formation and to define conditions inducing dermal-epidermal separation (DES).

Methods: In an ex vivo human model of BP, normal human skin cryosections were incubated with purified human peripheral blood eosinophils with or without activation in the presence or absence of BP autoantibodies, brefeldin A, diphenyleneiodonium (DPI), DNase, or blocking F(ab')2 fragments to CD16, CD18, CD32 and CD64. DES was assessed by light microscopy studies and quantified using Fiji software.

Results: Following activation with IL-5 and in the presence of BP autoantibodies, eosinophils induced separation along the dermal-epidermal junction of ex vivo skin. DES was significantly reduced by blocking any of the following: Fc**γ** receptor binding ($p=0.048$),

eosinophil adhesion (p=0.046), reactive oxygen species (ROS) production (p=0.002), degranulation (p<0.0001), or eosinophil extracellular trap (EET) formation (p=0.048).

Conclusions: Our results provide evidence that IL-5-activated eosinophils directly contribute to BP blister formation in the presence of BP autoantibodies. DES by IL-5-activated eosinophils depends on adhesion and Fc**γ** receptor activation, requires elevated ROS production and degranulation, and involves EET formation. Thus, targeting eosinophils may be a promising therapeutic approach for BP.

SIGLEC-7 DOWNREGULATES HUMAN EOSINOPHIL ACTIVATION IN VITRO

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Background: Siglec-7, Siglec-8 and CD300a are inhibitory receptors (IRs) expressed on the surface of human peripheral blood eosinophils. Whereas Siglec-8 and CD300a activation has been shown to downregulate eosinophil function, especially in allergic inflammatory responses, Siglec-7 expression and function on human eosinophils remain to be elucidated.

Methods: Eosinophil expression of Siglec-7 was measured by whole blood flow cytometry and quantitative PCR in hypereosinophilic patients (HES) and non-eosinophilic normal donors (ND). Expression of IRs on eosinophil surface (either whole blood or purified fraction) in the presence of either IL-5, GM-CSF (10ng/ml), Staphylococcus aureus enterotoxin B (SEB) or hypoxia (<3% O2) was quantified by flow cytometry. Eosinophil apoptosis was measured by AnnexinV-FITC/7-AAD staining. Eosinophil degranulation induced by GM-CSF (50ng/ml) after crosslinking of IRs using monoclonal antibodies (mAbs) and goat anti-mouse $F(ab')_2$ (10μg/ml) for 40 min or 18 hrs was assessed by measuring eosinophil peroxidase (EPO) and cytokine (TNFα and IL-8) release in the supernatants. Phosphorylation of SHP-1 and Erk1/2 and p38 MAPK were evaluated using Western Blot analysis.

Results: Siglec-7 expression on blood eosinophils was detected in all individuals tested (n=47 HES and 30 ND) and was significantly lower than that of CD300a or Siglec-8 (GM ΔMFI 235 vs 553 and 1131, respectively). Siglec-7 expression was increased on blood eosinophils from HES (n=38) compared to ND (n=31) (GM ΔMFI 102 vs. 218, p<0.01) and correlated with the absolute eosinophil count (r=0.33, P<0.01, n=72). Siglec-7 mRNA was measured in purified peripheral blood eosinophils from 6 ND and 19 HES. Levels were detectable in all subjects tested and were comparable between ND and HES. Siglec-7 expression on purified eosinophils was not significantly affected by stimulation with IL-5, GM-CSF, SEB or hypoxia, stimuli known to induce up-regulation of CD300a expression. Moreover, in contrast to anti-Siglec 8 mAbs, which induce eosinophil apoptosis in the absence of cross-linking after overnight priming with IL-5, eosinophil viability was unchanged in the presence of anti-Siglec-7 mAb (n=5). Importantly, in contrast to Siglec-8, both anti-Siglec-7 and anti-CD300a mAbs significantly inhibited GM-CSF-induced EPO and inflammatory mediator (TNFα and IL-8) release in vitro (n=8). Finally, Siglec-7 cross-linking on GM-CSF activated eosinophils, induced significant phosphorylation of SHP-1 (already after 30 seconds) and de-phosphorylation of Erk1/2 and p38 (after 5 and 15 minutes).

Conclusions: Siglec-7, Siglec-8 and CD300a are IRs that differ in their numbers of inhibitory motifs (ITIM) and expression levels on the eosinophil surface. Given the effect of Siglec-7 signaling on degranulation and inflammatory mediator release, targeting of Siglec-7 on eosinophils together with other IRs, such as Siglec-8 or CD300a, could lead to enhanced efficacy in the treatment of eosinophil-driven disorders.

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ADIPOCYTES SUPPORT EOSINOPHIL MIGRATION AND SURVIVAL

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Background: Recent data highlight fundamental functions for eosinophils in adipose tissue homeostasis. In fact, a new paradigm has emerged where resident adipose tissue eosinophils provide IL-4 to sustain the alternatively activated state of local macrophages which secrete catecholamines to promote metabolic homeostasis. Nonetheless, the factors, which mediate eosinophil migration and sustain their survival in the adipose tissue are unclear. Since eosinophils are found in substantial levels in the adipose tissue under steady state conditions, we hypothesized that adipocyte-derived mediators could support eosinophil migration and subsequent survival.

Methods: 3T3-L1 cells were differentiated in vitro into adipocytes and their culture media (CM) was obtained. Eosinophils were obtained from the peritoneal cavity of *Il5*^{Tg} mice and co-cultured with 3T3-L1 cells or in the presence of 3T3-L1 CM. mRNA expression

of CCL11, CCL24, IL-5, GM-CSF and IL-3 was determined in differentiated 3T3-L1 cells. Chemotaxis assays towards 3T3-L1 CM were performed using a 0.3μm trans-well system in the presence of anti-CCL11 neutralizing antibodies. Adipocytes were stimulated with IFN-γ or TNF-α and the secretion of CCL11 determined by ELISA. Wild type, *Il5*^{Tg} and ΔdblGATA mice were subjected to high fat diet (containing 60% fat). The mice were weighed once a week up to 16 weeks. Thereafter, the mice were subjected to glucose tolerance test and adipose tissue eosinophil and macrophage levels were determined using flow cytometry. Metabolic gene expression (e.g. *Ppara, Pparg, Leptin, Adiponectin, Cpt, Glut4, Fasn, Scd)* was determined using qPCR.

Results: Flow cytometric analysis of single cell suspensions from the adipose tissue of naïve wild type mice revealed that eosinophils comprise a substantial portion of all CD11b^{+} myeloid cells. Adipose tissue eosinophil levels were decreased and macrophage levels increased following feeding with high fat diet. Assessment of various metabolic genes in the adipose tissue of naïve ΔdblGATA mice revealed markedly decreased mRNA expression of *Ppara, Pparg, Leptin, Adiponectin, Cpt, Glut4, Fasn, Scd* but not *Fabp4*. Importantly, *Il5*^{Tg} mice that were fed with high fat diet displayed significantly attenuated weight gain that was accompanied with decreased glucose intolerance.

To assess the factors regulating baseline eosinophilia in the adipose tissue, 3T3-L1 cells were differentiated and their CM obtained. 3T3-L1 cell CM was capable of promoting eosinophil chemotaxis and survival a finding that was recapitulated in also in co-cultures of primary adipose tissue with eosinophils. 3T3-L1 cells displayed readily detectable mRNA levels of CCL11, IL-5, GM-CSF and IL-3. Furthermore, IFN-γ and TNF-α stimulated 3T3-L1 cells to secrete CCL11 and neutralization of CCL11 abrogated 3T3-L1 CM-induced eosinophil migration.

Conclusions: We show that eosinophils have a key role in adipose tissue homeostasis and that adipocytes can attract eosinophil migration and survival. Collectively, understanding the molecular pathways, which regulate the accumulation and survival of eosinophils has potential implications at preventing and/or treating disorders of metabolism such as obesity.

THE ROLE OF AUTOPHAGY IN EOSINOPHILS

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Background: Autophagy is a highly regulated catabolic process in which cell constituents are targeted for lysosomal degradation, partly in order to assure energy and precursor molecules for cell survival. The autophagic pathway is incorporated in various biological mechanisms including multiple innate and adaptive immune pathways. Our research group demonstrated that this pathway negatively affects neutrophil generation and is also involved in lipid metabolism, where it is responsible for degrading lipid droplets in murine hepatocytes.

Methods: To study the role of autophagy in eosinophils, we generated an eosinophil-specific *Atg5* knockout mice (designated *Atg5*^{EA}). Because eosinophils in mice comprise only 1-3% of circulating leukocytes, we provoke eosinophilia by crossbreeding our genetically modified mice with IL-5 transgenic mice.

Results: ATG5 was efficiently and specifically deleted within the eosinophilic lineage and these cells were autophagy deficient. We measured eosinophil numbers and discovered decreased absolute (p=0.0085) and relative (p=0.0271) numbers in the peripheral blood of *Atg5*^{EA} IL-5 transgenic mice as compared with control IL-5 transgenic mice. We found that Atg5^{EA} IL-5 transgenic mice have greater numbers of immature eosinophils than counterpart controls (mean relative numbers of Siglec-F^{int}CCR3^{-} cells: bone marrow, 32.9% *versus* 24.8%; spleen, 21.5% *versus* 12.9%). Lack of *Atg5* does not compromise the viability for *in vitro* culture of eosinophils isolated from bone marrow. Furthermore, in *Atg5*-deficient eosinophils, we detected increased numbers of lipid bodies as compared with control eosinophils (p=0.0004). Upon physiologic stimulation with C5a following GM-CSF priming, however, eosinophils deficient for *Atg5* also exhibit an impaired ability for *de novo* synthesis of lipid bodies.

Conclusions: Decreased numbers of *Atg5*-deficient eosinophils in peripheral blood and higher numbers of immature *Atg5*-deficient eosinophils at sites of eosinophilopoesis suggest that the autophagic pathway is required for normal eosinophil maturation. Furthermore, increased numbers of lipid bodies in eosinophils deficient for *Atg5* imply the involvement of the autophagic pathway in lipid regulation.

HAEMOSPHERE: A WEB PORTAL TO DELIGHT IMMUNOLOGISTS AND HAEMATOLOGISTS ALIKE

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Background: Haematopoiesis is a multi-stage process involving the differentiation of stem and progenitor cells into distinct mature cell lineages.

Methods: Here we present our data set: Haemopedia, an atlas of murine gene expression data containing 54 haematopoietic cell types, covering all the mature lineages in haematopoiesis. And an online web portal to interegate this and other data sets: Haemosphere, to make analyses of Haemopedia and other blood cell transcriptional datasets easier. This resource provides simple tools to interrogate gene expression-based relationships between haematopoietic cell types and genes of interest. We include rare cell populations such as eosinophils, mast cells, basophils and megakaryocytes and a broad collection of progenitor and stem cells.

Results: We show that lineage branching and maturation during haematopoiesis can be reconstructed using the expression patterns of small sets of genes. We also identify genes with enriched expression in each of the mature blood cell lineages. Many of which show conserved lineage-enriched expression in human haematopoiesis. We identify genes that are expressed selectively and highly in specific lineages. The eosinophil lineage displayed the smallest signature with just 49 uniquely upregulated probes, including genes encoding eosinophil granule proteins such as *Ear2* and *Prg3*, while sharing some common pathways with the other granulocytes in the atlas.

In addition to providing access to Haemopedia, Haemosphere includes a selection of other published datasets considered as haematopoietic "atlases". Haemosphere offers:

- Interactive expression profile plots for genes of interest.
- Analysis of gene set expression in a selected dataset through the generation of an interactive heatmap.
- Interactive multidimensional scaling plot enables visualization of relationships between cell types within a dataset.
- Identification of differentially expressed genes between two cell types or lineages within a dataset using well established methods (limma from R/Bioconductor)
- Finding correlated and negatively correlated genes.
- Linking genes to their orthologues.

Haemopedia includes transcription profiles from rare haematopoietic cells types such as eosinophils, for which published transcription datasets are relatively scant. To illustrate the combined power of Haemopedia and Haemosphere, we performed an example analysis using the tools we provide in Haemosphere, which yielded a novel eosinophil specific transcription factor: *Mkx*. We generated an Mkx-driven green fluorescent protein (GFP) reporter mouse, MkxGFP. The high expression of *Mkx* observed in eosinophils was derived from an *Mkx* positive subpopulation. Peritoneal cavity eosinophils were shown to have the largest proportion of *Mkx* positive cells, followed by eosinophils from the spleen and peripheral blood, and then the bone marrow.

Conclusions: Haemopedia, is a comprehensive collection of gene expression data covering all haematopoietic lineages, which, along with Haemosphere (a web portal and suite of analysis tools), will enable improved understanding of the molecular and genetic regulation of blood cell function and production.

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CHARACTERIZING A LEUKOCYTE-SPECIFIC RHO GUNAINE NUCLEOTIDE EXCHANGE FACTOR PROTEOFORM IN EOSINOPHILS

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Background: Three RhoA GTPase-regulating guanine nucleotide exchange factor (ARHGEF) proteins are abundant in eosinophils: ARHGEF1, 2 and 18. RhoA is a key regulator of cell shape change through actin remodelling, and thus investigations of a novel proteoform of one of these exchange factors found only in leukocytes may lead to further insight into leukocyte-specific events such as the profound morphological change of activated eosinophils.

Methods: Initial data were acquired from a quantitative proteomics investigation of resting blood eosinophils: two-dimensional liquid chromatography in conjunction with mass spectrometry yielded peptides mapping to 6,813 genes (Wilkerson *et al*., J Pro-

teomic Res, 2016). One of these presumptive genes, encoding a protein annotated as LOC100996504 in UniProt, was further investigated. We amplified and sequenced eosinophil cDNA, immunoblotted eosinophil lysates, and used bioinformatics tools to validate predicted sequence. We localized ARHGEFs 1, 2 and 18 in eosinophils using antibody-staining and microscopy.

Results: Exploration of genomic, transcriptomic and proteomic databases indicated that LOC100996504 is not the discrete protein described in UniProt but rather the N-terminal domain of a 1361-residue ARHGEF18 proteoform dubbed LOCGEF. This proteoform arises from leukocyte-specific use of an alternative start site. The existence of LOCGEF in eosinophils was confirmed by cloning of its cDNA from eosinophils and identification of the overlapping sequence as a peptide sequence match in the proteomic dataset. Inasmuch as the relative abundances of LOC100996504 and ARHGEF18 varied by less than 30% and only a band of 150-kDa was detected by immunoblotting of eosinophil lysates with anti-ARHGEF18, LOCGEF appears to be the only proteoform of ARHGEF18 in eosinophils, i.e., the 114-kDa proteoform ubiquitous in other tissues was not identified in eosinophils. The three ARHGEFs displayed different behavior during the profound morphological change accompanying eosinophil activation. Immunostaining revealed that ARHGEF18 relocalized from the periphery of round unstimulated eosinophils to the nucleopod of polarized interleukin-5-treated eosinophils after activation for 5 min and remained there at 60 min. In contrast, ARHGEF2 localized to the nucleopod after 5 min of activation but had moved to the granulomeric region at 60 min, and ARHGEF1 was in the granulomeric region at both time points.

Conclusions: The heretofore unknown LOCGEF proteoform of ARHGEF18 has been characterized in eosinophils. The immunostaining results suggest that the three ARHGEFs that regulate RhoA, including LOCGEF, have specialized functions in activated polarized eosinophils.

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OP#1 BENRALIZUMAB IS A WELL-TOLERATED AND EFFECTIVE TREATMENT FOR *PDGFRA*-NEGATIVE HYPEREOSINOPHILIC SYNDROME

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Rationale: Conventional treatment options for hypereosinophilic syndromes (HES), including corticosteroids, cytotoxic and immunomodulatory drugs, have variable efficacy and significant toxicity. Benralizumab is an afucosylated monoclonal antibody that binds IL5Rα expressed on eosinophils, targeting them for antibody-dependent cell death.

Methods: Twenty *PDGFRA*-negative adult subjects with varied clinical subtypes of HES and absolute eosinophil count (AEC) ≥1000 cells/mm^3 on stable background therapy were enrolled on a two stage clinical trial of benralizumab (NCT02130882). During the placebo-controlled, double-blind stage, subjects were randomized to 3 monthly doses of benralizumab (30 mg sc) or placebo while on stable background therapy. AEC was blinded during this time. At week 12, subjects entered the open-label stage and received benralizumab. AEC was unblinded beginning at week 13. Depending on the results of the week 13 AEC, subjects were eligible to continue to receive monthly benralizumab with tapering of background therapy. Clinical and laboratory evaluations were performed at every visit. Bone marrow biopsy was performed at baseline and 12 weeks. Biopsies of affected tissues were obtained at baseline or during screening and at 24 weeks. The primary endpoint was the proportion of subjects with a 50% decrease in AEC at week 12. Secondary endpoints included the frequency and severity of adverse events, reduction in signs and symptoms of HES and improvement in tissue eosinophilia.

Results: Subject enrollment is complete and 18/20 subjects have reached the open-label stage (1 withdrawal, 1 subject still in placebo-controlled stage). Median baseline AEC at enrollment was 1845/mm^3 (range 1160-26420). The week 12 AEC results remain blinded until the last subject completes the placebo-controlled stage. Week 13 AECs decreased to <50% of baseline in 16/18 evaluable subjects (median AEC 0/mm^3; range 0-160). AEC was unchanged from baseline in 2 subjects, both of whom carry JAK2 mutations. Eleven of the 16 responders reported improvement in HES symptoms and were able to taper background therapy. Biopsies of affected tissues were performed in 7 subjects and showed resolution of tissue eosinophilia at week 24. Eosinophilia and symptoms returned between weeks 24 and 32 in three lymphocytic variant HES subjects with CD3-CD4+ aberrant T cell populations. The remaining 13 subjects remain on benralizumab for a median of 16±3 months (range 4-32). The drug has been well-tolerated with the exception of mild to moderate symptoms (fever, chills, nausea, or myalgias) that occur 6-8 hours after drug administration, and last <24 hours. These occurred in 8 subjects following the first dose of drug vs. placebo or the first dose of open-label benralizumab. There have been six serious adverse events, only one of which (a ureteral stone) was felt to be possibly related to benralizumab.

Conclusions: Preliminary data from this randomized, double-blind, placebo-controlled trial suggest that benralizumab is a well-tolerated and effective treatment for most *PDGFRA*-negative HES.

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OP#2 EOSINOPHIL PEROXIDASE INCREASES THYMIC STROMAL LYMPHOPOIETIN EXPRESSION IN KERATINOCYTES

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Background: Eosinophils play an important role in mediating itch in atopic dermatitis, and we have recently found that the eosinophil granule protein eosinophil peroxidase (EPX) is required for itch in a mouse model of atopic dermatitis. The cytokine thymic stromal lymphopoietin (TSLP) activates sensory nerves, inducing itch. We tested whether EPX is required for increased TSLP in a mouse model of atopic dermatitis and is sufficient to induce TSLP expression in keratinocytes *in vitro*.

Methods: Primary keratinocytes were isolated from mouse tail skin and were treated with either 1) EPX (30nM; isolated from human eosinophils), plus its substrates hydrogen peroxide (100μM) and bromide (100μM), 2) EPX alone, or 3) substrates alone. Cells were treated for 1 to 24 hours in the absence and presence of peroxidase inhibitor resorcinol (30μM). Gene expression for TSLP was analyzed using real-time RT PCR, and TSLP levels in media were measured using an ELISA. *In vivo* experiments were carried out using trimellitic anhydride (TMA) to induce atopic dermatitis in mouse ears. WT BALB/c and EPX knockout mice were sensitized to

TMA, and challenged for the following 9 days by applying TMA to the ears. Ears were harvested and immunostained for TSLP after final challenge. Animal protocols were approved by the institution IACUC.

Results: EPX and its substrates significantly increased TSLP gene expression in keratinocytes, and significantly increased TSLP secretion. Neither EPX alone nor its substrates had any effect. The effect of EPX and its substrates on TSLP gene expression was abolished in the presence of the peroxidase inhibitor resorcinol. In the TMA induced atopic dermatitis model cutaneous TSLP immunostaining was increased in WT mice, but not in EPX knockout mice.

Conclusions: These data show that EPX induces expression of TSLP in an atopic dermatitis mouse model and in cultured keratinocytes, and that this requires the peroxidase activity of EPX. Our results suggest that eosinophils mediate itch in atopic dermatitis by increasing TSLP in skin and eosinophil peroxidase may be an effective therapeutic target to treat itch in atopic dermatitis.

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OP#3 LYMPHOCYTIC VARIANT HYPEREOSINOPHILIC SYNDROME: DIAGNOSTIC TOOLS REVISITED

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Background: Lymphocytic variant hypereosinophilic syndrome (L-HES) is a rare disorder that is often under-recognized, even in tertiary centers.

The objective of this study is to provide guidelines for diagnosis of L-HES, through assessment of the discriminative value of currently available diagnostic tools, with regard to other causes of hypereosinophilia.

Methods: 105 patients with hypereosinophilia were included in this study (20 L-HES associated with CD3-CD4+ T-cells, 13 idiopathic systemic HES, 31 organ-restricted HES, 5 HEUS, 36 hypereosinophilia with a known underlying disease). Results of lymphocyte phenotyping (CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD27, CD45RO, CD95, HLADR), PCR analysis of TCRβ/γ gene rearrangements, serum biomarker (IgE, IgG, IgM, TARC) measurements, intracytoplasmic cytokine (IFNγ, IL2, IL5, IL4, IL13) detection and production by PMA-ionophore-stimulated PBMC were compared between L-HES patients versus other symptomatic patients with hypereosinophilia using Mann Whitney and Kruskall Wallis tests.

Results: Among 20 patients with L-HES, 7 (35%) had ≤5% CD3-CD4+ cells among circulating lymphocytes, among which 4 had ≤1%. Flow cytometry allowed confident detection of these minute subsets, with significantly different staining intensity for CD2 (higher MFI than CD3+CD4+ cells, p 0.0003), homogenous positivity for CD45RO and CD95, and lower expression of CD7 and CD27. Demonstration of a clonal TCR rearrangement in blood is neither sensitive (63%) nor specific (67%) for L-HES. Serum IgE levels were not significantly higher in L-HES patients, whereas IgM levels were (p 0.0004).

The proportion of CD3-CD4 T-cells producing IL5 (mean 29%, range 4.5-67.7%), IL4 (mean 44%, range 24.7-60.5%), and IL13 (mean 54%, range 32.5-74%) was significantly higher than CD3+CD4+ cells from other patients with symptomatic hypereosinophilia. (mean, range : IL-5 3%, 0.5-6.3%; IL-4 6%, 1.7-13.3%; IL-13 4%, 1.3-14.1%) and from healthy controls (p <0.005). However, IGNγ was detectable in >10% of CD3-CD4+ cells in more than half of L-HES subjects, making its absence an unreliable marker for this variant.

Although significantly higher IL-5 and IL13 levels were found in stimulated PBMC culture supernatants from L-HES patients, heterogeneity of results among groups was such that this tool provides no added value.

The serum TARC level was significantly higher for L-HES compared to other hypereosinophilic patients (p <0.0001). A ROC curve showed that at 3273 pg/ml, the sensibility and specificity of this test for L-HES is 90 and 80% respectively (positive likelihood ratio of 4.5). HES patients with TARC above this threshold, but no detectable CD3-CD4+ cells, did not differ from those with normal TARC levels in terms of serum IgE levels, intracytoplasmic or secreted Th2 cytokines, or T cell clonality.

Conclusions: Diagnosis of L-HES associated with CD3-CD4+ T-cells can accurately be made on the basis of an extended phenotyping panel including CD2, CD45RO and CD95, together with demonstration of intracytoplasmic IL-4 and IL-13 in >25% of abnormal cells. For individual patients with symptomatic hypereosinophilia, TCR gene rearrangement and serum TARC levels are of little help in distinguishing those with L-HES. Although no evidence for an underlying Th2 disease in non-L-HES patients with high TARC was found in peripheral blood, studies are ongoing to further explore this possibility and potentially develop new diagnostic tools for T-cell driven HES.

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OP#4 SIGLEC-8 IS AN ACTIVATING RECEPTOR ON HUMAN EOSINOPHILS FOR INTEGRIN-DEPENDENT ADHESION, ROS GENERATION AND APOPTOSIS

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Background: Siglecs (sialic acid-binding, immunoglobulin-like lectins) are type I transmembrane proteins expressed primarily on leukocytes that have been shown to negatively regulate cell function. Among them is Siglec-8, a CD33 subfamily member that is selectively expressed on the cell surface of human eosinophils. Siglec-8 has an intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM), putatively responsible for signal transduction. Engagement of Siglec-8 causes resting eosinophil apoptosis in a caspase-dependent manner. In cytokine-primed eosinophils, Siglec-8 binding causes apoptosis with increased mitochondrial damage and reactive oxygen species (ROS) production, but exact signaling mechanisms are unknown.

Methods: Using a mAb (2C4) against Siglec-8, we first examined Siglec-8-mediated phosphorylation of signaling molecules in the presence or absence of IL-5 priming using phosphoproteomics analysis. In follow-up experiments, we used blocking antibodies, pharmacological inhibitors and western blot analysis to fully understand the necessity of the identified phosphoproteins in Siglec-8 function on human eosinophils.

Results: We observed that Siglec-8 engagement on cytokine-primed eosinophils promoted β2-integrin dependent eosinophil spreading and adhesion, and that adhesion was necessary for subsequent ROS generation and apoptosis. Additional experiments demonstrated that Siglec-8-mediated ROS was generated via NADPH oxidase activation, because pretreatment of eosinophils with catalase (an extracellular superoxide scavenger) or NSC23766 (a Rac1 GTPase inhibitor) completely inhibited Siglec-8-mediated eosinophil apoptosis. Finally, engagement of Siglec-8 on cytokine-primed eosinophils resulted in increased phosphorylation of Akt, p38 and JNK1, and pharmacologic inhibition of these pathways prevented Siglec-8-mediated eosinophil apoptosis.

Conclusions: We demonstrate for the first time that Siglec-8 functions as an activating receptor on cytokine-primed eosinophils via positive regulation of β2-integrins, NADPH oxidase and a subset of protein kinases.

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OP#5 CD300F INHIBITS ADIPOSE TISSUE EOSINOPHIL HOMING AND SUBSEQUENT IL-4 PRODUCTION BY REGULATING IL-5 RECEPTOR SIGNALING

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Background: Adipose tissue eosinophils have a central function in the regulation of adipose tissue homeostasis. Recent data established key roles for IL-5, eosinophil-derived IL-4 and alternatively activated macrophages (M2) in this process. Nonetheless, molecular pathways that regulate the accumulation of eosinophils in the adipose tissue and their subsequent release of IL-4 are unknown. CD300f is a cell surface receptor belonging to the Ig-superfamily, which under baseline conditions, is expressed in the adipose tissue (AT) nearly exclusively by eosinophils. Thus, we hypothesized that CD300f will regulate the activities of adipose tissue eosinophils.

Methods: The expression of CD300f on bone marrow (BM)-derived eosinophils as well as in eosinophils from WT, *Il5^{tg}* and *Il5$^{-/-}$* mice was determined by flow cytometry. *Il5^{tg}/Cd300f$^{-/-}$* mice were generated and baseline parameters including histology, cell infiltration, M2 macrophage genes and IL-4 secretion were measured (qPCR and ELISA). Microarray analysis was performed on BM and AT eosinophils from *Il5^{tg}* and *Il5^{tg}/Cd300f$^{-/-}$* mice. CD300f ligand expression was assessed using CD300f-fc-fusion protein staining. Metabolic parameters were compared between WT, *Cd300f$^{-/-}$, Il5^{tg}* and *Il5^{tg}/Cd300f$^{-/-}$* mice under high fat diet regime. Finally, regulation on IL-5 receptor signaling was studied by over expression of CD300f in I.29 B cells (phosphoflow, western blot).

Results: CD300f was exclusively expressed by eosinophil progenitors and regulated by IL-5. Generation of *Il5^{tg}/Cd300f$^{-/-}$* mice revealed that marked and distinct increase in eosinophil levels in the white and brown adipose tissue but not spleen, peritoneal cavity and GI tract in comparison with WT, *Cd300f$^{-/-}$*, and *Il5^{Tg}* mice. Increased eosinophilia in the adipose tissue of *Il5^{tg}/Cd300f$^{-/-}$* mice was accompanied with substantially increased IL-4 production. Indeed, CD300f regulated IL-5-driven ERK and AKT activation and negatively regulated by IL-4 production from eosinophils in response to local AT factors. Consequently, increased IL-4 production resulted in increased proliferation of monocytes and elevated Arginase1, YM1, Relm-α and eotaxin-2 expression by M2 macrophages. Interestingly, AT but not lung endothelial cells expressed a CD300f ligand possibly explaining increased eosinophilia spe-

cifically in the AT. *Cd300f*$^{-/-}$ mice displayed age-related accumulation of eosinophils and macrophages and decreased adipose tissue weight, which was associated with decreased diet-induced weight gain and insulin resistance. Importantly, *Il5*^{Tg}*/CD300f*$^{-/-}$ were completely protected from diet-induced weight gain and glucose intolerance.

Conclusions: We show here that IL-5 dependent homing and activation of AT eosinophils is negatively regulated by CD300f via a ligand expressed by local epithelial cells. These findings highlight CD300f as a novel regulator of AT tissue eosinophils and offer new cellular checkpoints, which could be further developed in the global battle against obesity and its complications.

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OP#6 EOSINOPHILS STIMULATE AIRWAY SENSORY NERVE GROWTH IN ASTHMA

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Background: Airway nerves control bronchoconstriction. In asthma, eosinophils heighten airway responses in part by altering nerve function, but their effects on airway nerve structure are unknown.

Hypothesis: Eosinophils increase airway responsiveness by stimulating sensory nerve growth.

Methods: Bronchoscopic airway biopsies were obtained from the right middle lobe carina of asthmatics and non-asthmatic subjects. The protocol was IRB approved and written informed consent was provided. Nerves and eosinophils were immunolabeled with antibodies to PGP9.5 and eosinophil peroxidase, respectively. Whole-mount images were acquired using an LSM780 confocal microscope (63X, 1.4 NA). Nerve morphology (branch points, neurite length) and eosinophils were quantified 3-dimensionally in the airway epithelium and subepithelium (Imaris). Transgenic mice with airway eosinophilia (IL5tg), eosinophil-deficient mice (PHIL), eosinophil-deficient mice with elevated IL-5 (IL5tg-PHIL) and C57BL/6 wild-type (WT) mice were sedated, paralyzed and ventilated. Airway resistance was measured in response to serotonin (5-HT; 1-100 mM IV) and airway nerves were quantified as described above. The Animal Care and Use Committee approved all protocols.

Results: Thirty-one subjects with persistent asthma (GINA treatment stage 2-5), 13 with intermittent asthma (GINA stage 1) and 19 non-asthmatic controls were included in the analysis. Age, body-mass index and gender distribution were similar between groups. Persistent asthmatics had lower FEV1's and worse asthma quality of life questionnaire scores. Nerve length and branch points were significantly increased in persistent asthmatics compared to subjects with intermittent asthma and non-asthmatic controls. Greater nerve length correlated with increased airway eosinophils in persistent, but not intermittent asthma. Despite elevated blood eosinophils in persistent asthmatics overall, blood eosinophil counts did not correlate with tissue eosinophils within individual subjects. In mice, airway eosinophils increased nerve length and branching (IL5tg), and caused neuronally-mediated airway hyperresponsiveness to 5-HT compared to WT mice, eosinophil-deficient PHIL mice and eosinophil-deficient mice with elevated IL-5 (IL5tg-PHIL).

Conclusions: Eosinophils stimulate airway sensory nerve growth that contributes to nerve-dependent airway hyperresponsiveness. Eosinophils' effects on airway nerves have an underappreciated role in asthma.

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#1 COMPOSITION OF EXTRACELLULAR MATRIX MICROENVIRONMENT DICTATES PHENOTYPIC PLASTICITY, *IN SITU* EXPANSION, MATURATION, AND SURVIVAL OF TISSUE EOSINOPHILS

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Despite multiple strategies seeking to deplete eosinophils at sites of allergic inflammation, little is known about local tissue factors regulating survival and *in situ* expansion of eosinophils in the lung. Using a systems biology approach, we contrasted phenotypes and function of tissue eosinophil subsets against transcriptomics profiles of the lung in three different tissue states: homeostasis, development, and allergic disease. This led us to hypothesize that the morphogenetic composition of the lung extracellular matrix (ECM), present during normal development and recapitulated in allergic disease, has a key potential to alter the phenotype and function of resident/recruited eosinophils. Specifically, tenascin C (TNC), a provisional extracellular matrix protein deposited only during epithelial repair/remodeling, showed a strong association with accumulation of tissue eosinophils in normal development and allergic disease. *In vitro* assays utilizing bioengineered matrix scaffolds showed that eosinophils interacting with a matrix environment representing tissue morphogenetic/remodeling states (enriched with provisional glycoproteins) exhibit significantly enhanced haptotactic and chemotactic responses, when compared to basic composition ECM representing homeostasis. RNA-Seq analysis of naïve mouse eosinophils interacting with TNC-enriched provisional matrix identified a 1621-gene signature representing enhanced degranulation, adhesion and altered metabolism. This is significant when compared to only 763 eosinophil genes induced by interaction with homeostatic ECM. Uniquely, naïve murine eosinophils exposed to TNC significantly upregulated markers of immature eosinophils (CD34, CD117, CD125 and Sca-1) and anti-apoptotic genes. We hypothesized that TNC also regulates survival and expansion of eosinophil progenitors, which we tested kinetically in bone marrow-derived eosinophil cultures (10 ng/ml Il-5 with +/- 25 ug/ml TNC). We found that TNC: 1) expanded the available Lin(-)Sca1(+) early precursor pool 3-fold; 2) significantly downregulated expression of IL-5Ra during the eosinophil lineage commitment phase; 3) block of eosinophil maturation by TNC was reversible, and its withdrawal from cultures increased final mature eosinophil yield; 4) enhanced survival of Lin(+/-)Siglec-F(+) Sca-1(+) immature eosinophils. Remarkably, gene expression signatures of naïve eosinophils activated by interaction with TNC-enriched ECM *in vitro* (in absence of cytokine or chemokine stimulation) and eosinophils sorted from murine lungs in an ovalbumin model of asthma showed 30% alignment, completely agreeing on expression of key markers of tissue-activated eosinophil subsets: Car4, CD34, Itgax (CD11c), Itgae (CD103), Itgb5, CD44, Siglec-F, Tlr4, Mmp9. Collectively, our results implicate significant non-inflammatory potential of local tissue factors to promote *in situ* expansion and persistence of eosinophilia in allergic disease.

#2 EOSINOPHILS AND MORPHOLOGICAL REMODELING OF THE ESOPHAGEAL EPITHELIUM IN EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) is a recently emerging chronic inflammatory disease of the esophagus and is mediated by dietary food antigens and clinically characterized by upper gastrointestinal (GI) symptoms including dysphagia and food impaction. Histopathological manifestations include intraepithelial eosinophilic inflammation and alterations of the esophageal epithelium such as basal layer hyperplasia (BZH) and dilated intercellular spaces (DIS), which are thought to drive the substantial esophageal dysfunction of EoE. The underlying immune pathways that drive the individual pathological manifestations of disease remain largely unexplored.

Results: We recently used an integrated approach interfacing bioinformatics outcomes derived from genetic profiling (RNA sequencing [RNA-Seq], miRNA, and gene expression array analyses) of pediatric EoE cohorts and identified altered expression of ion transport genes (*SLC9A3, SLC26A4, CAII*) that are integrally involved in the acid-base transport circuit of the esophagus. Employing microscopy imaging technologies and electrophysiological analyses of pediatric EoE biopsy samples and primary and immortalized esophageal cells we have identified dysregulation of the acid-base transport circuit in esophageal cells was associated with altered acid (H^+) and base (HCO_3^-) ion secretion. We show that these acid-base transport circuits are important in BZH and DIS formation.

Conclusions: Collectively, these data identify a functional role for acid-base transport circuit in esophageal cell proliferation and BZH in EoE, and provides rationale for pharmacological intervention of acid-base transport circuit in EoE.

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#3 COMPARTMENTALIZATION AND VESICULAR TRAFFICKING OF INTERFERON-GAMMA (IFN-γ) WITHIN HUMAN EOSINOPHILS

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Background: Eosinophils are able to release numerous mediators that are pre-synthesized and stored within their cytoplasmic specific (secretory) granules. High levels of interferon-gamma (IFN-γ) are constitutively expressed in human eosinophils, but the intracellular compartments involved in the transport and release of this cytokine remain to be established. In this work, we investigated the ultrastructural localization of IFN-γ in human eosinophils stimulated or not with inflammatory mediators.

Methods: A pre-embedding immunonanogold transmission electron microscopy (TEM) technique that combines optimal epitope preservation and secondary Fab-fragments of antibodies linked to 1.4 nm gold particles for optimal access to membrane microdomains were used to investigate IFN-γ subcellular localization in human eosinophils from healthy donors stimulated or not with tumor necrosis factor alpha (TNF-α) or with the CC-chemokine ligand 11 CCL11 (eotaxin-1). In parallel, eosinophils were also prepared for conventional TEM to study the morphological patterns of secretion. Quantitative TEM was applied to evaluate the number of large transport carriers (Eosinophils Sombrero Vesicles – EoSVs) and their distribution in the cytoplasm.

Results: Both CCL11 and TNF-α induced morphological patterns of eosinophil secretion. Cells stimulated with CCL11 showed piecemeal degranulation (PMD), characterized by cytoplasmic vesiculation and progressive granule emptying. TNF-α triggered PMD and mainly compound exocytosis, characterized by granule-granule fusion. Quantitative TEM revealed that both CCL11 and TNF-α-activated eosinophils significantly increased the numbers of EoSVs compared to the unstimulated group (24.5 ± 3.00 for unstimulated; 55.1 ± 6.63 for CCL11 and 56,1 ± 4.42 for TNF-α; mean ± SEM, n= 1357 EoSVs counted in 30 cell sections), indicating that this vesicular system is actively formed in response to cell activation. Ultrastructural immunolabeling consistently detected IFN-γ on secretory granules (outer membranes and matrices) and on EoSVs in both unstimulated and stimulated cells. Labeling was clearly associated with vesicle membranes. The total number of IFN-γ-positive EoSVs was significantly higher in stimulated compared to unstimulated cells (8.60 ± 1.51; 20.30 ± 2.72; 23.50 ± 2.76 for unstimulated, CCL11 and TNF-α groups, respectively, mean ± SEM, n= 524 EoSVs). Moreover, EoSVs carrying INF-γ had a differential distribution in the cytoplasm of activated cells, being significantly higher in the cell periphery (1µm wide from the plasma membrane) compared with the cell inner (the contiguous cytoplasmic area deeper in the cell). This means that IFN-γ mobilization is associated with vesicular transport.

Conclusions: Our results provide direct evidence that human eosinophils compartmentalize IFN-γ within secretory granules and identify, for the first time, a vesicular trafficking of IFN-γ associated with large transport carriers. This is important to understand how IFN-γ is trafficked and secreted during inflammatory responses.

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#4 ROCKs NEGATIVELY REGULATE SECRETION OF EOSINOPHIL ASSOCIATED RNASES BY AFFECTING CD11b INTEGRIN

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Background: Eosinophil cytoplasmic granules contain preformed cationic cytotoxic proteins that are not found elsewhere. Among these proteins are eosinophil-associated RNases (EARs), which have been shown to be involved in host defense, tissue remodeling, immunity regulation and pathology in eosinophil-associated diseases. Rho-associated coiled-coil forming kinases (ROCK) are known downstream effectors of Rho A, and are involved in cytoskeletal reorganization, stress fiber and focal adhesion formation. ROCK inhibition was found to inhibit eosinophil chemotaxis and migration into lung during airway inflammation. However, ROCKs role in degranulation from eosinophils was not addressed. This study aimed to examine the role of ROCKs in EARs secretion.

Methods: ROCKI and II involvement in CCL11-stimulated EAR secretion were examined by treatment of human or mouse eosinophils with the highly potent and selective cell permeable inhibitor Y27632, that blocks ROCKI and II with similar potencies. EAR secretion was assessed by measuring RNase activity of secreted granule-associated RNases. Integrin expression was performed by flow cytometry.

Results: Effective concentrations of Y27632, that blocked chemotaxis of murine and human eosinophils, did not inhibit CCL11-mediated degranulation and even significantly increased it (x1.5) compared to vehicle. Moreover, degranulation enhancement by Y27632 was not due to an effect on actin polymerization or surface expression of CCL11 receptor, CCR3. In addition, the inhibitor Y27632 also increased spontaneous EAR secretion. It was previously shown by our studies and others that beta-2 integrin mediated

spreading is essential for CCL11-mediated degranulation and EAR secretion of eosinophils. Along these lines, we found that Y27632 pretreatment of human eosinophils significantly increased the surface expression of CD11b, as well as its active conformation in the presence and absence of CCL11. Similar results with CD11b surface expression were also found with mouse eosinophils.

Conclusions: Our results suggest that ROCKs are playing a negative regulatory role in EAR secretion of human and mouse eosinophils by controlling CD11b expression and activation state.

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#5 CHARACTERIZATION OF A NOVEL MOUSE STRAIN EXPRESSING HUMAN SIGLEC-8 ONLY ON EOSINOPHILS

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Background: Sialic acid-binding immunoglobulin-like lectin (Siglec)-8 is a human cell surface protein expressed exclusively on eosinophils, mast cells, and basophils that, when engaged, induces eosinophil apoptosis and inhibits mast cell mediator release. This makes Siglec-8 a promising therapeutic target to treat diseases involving these cell types. However, pre-clinical studies of the effects of Siglec-8 targeting in vivo are lacking due to the fact that this protein is only found in humans, apes, and some monkeys. Therefore, we have developed a new Siglec-8 knock-in mouse strain.

Methods: The *SIGLEC8* gene and an upstream loxP-flanked STOP cassette were introduced by homologous recombination into the *Rosa26* locus. When crossed with mice expressing Cre recombinase driven by the eosinophil peroxidase promoter (*eoCre*), the STOP cassette is removed and Siglec-8 is expressed in these cells. To initially characterize this new strain, we examined Siglec-8 surface expression, endocytosis, and signaling in mouse eosinophils from various tissues and those differentiated in vitro from bone marrow.

Results: We confirm that the Siglec-8 protein is expressed on the surface of mature eosinophils and only on eosinophils in multiple tissue compartments at levels comparable to those seen on human blood eosinophils. No changes in baseline blood or tissue eosinophil numbers were evident compared to littermate controls. Using eosinophils derived from bone marrow of these mice, we demonstrate that, during maturation, Siglec-8 surface expression occurs after Siglec-F but before the appearance of the late eosinophil developmental marker CCR3, consistent with the timing of eosinophil peroxidase expression during eosinophil development. In response to antibody ligation, Siglec-8 is endocytosed more rapidly than Siglec-F, and this ligation alters the phosphotyrosine profile of these cells, indicative of ligand-induced signaling. Finally, employing a model of ovalbumin sensitization and airway challenge, we show that this Siglec-8 knock-in mouse strain generates a similar pattern of allergic lung inflammation when compared to littermate controls.

Conclusions: This new mouse strain should be a suitable tool to investigate Siglec-8 targeting in vivo in a variety of eosinophilic disease settings. By enabling more facile manipulation compared to human eosinophils, this mouse strain may also shed new light on other aspects of Siglec-8 biology.

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#6 CHARACTERIZING A NOVEL PROTEIN IN EOSINOPHILS: NANCE-HORAN SYNDROME-LIKE PROTEIN 2 (NHSL2)

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Background: NHSL2 (Nance-Horan Syndrome-like protein 2) is a poorly-characterized protein present in eosinophils. It contains regions with sequence identity to Nance-Horan Syndrome (NHS) protein, mutations of which are associated with abnormal craniofacial development. Both genes are located on the X chromosome. Nothing has been published on the cellular function of NHSL2. NHSL2 is highly-conserved, with homologs in *Xenopus* and zebrafish. Our attention was drawn to NHSL2 in the proteomics dataset because of its many-fold increases in phosphorylation at multiple sites in interleukin 5 (IL5)-activated eosinophils.

Methods: The proteomics datasets were acquired in an investigation of resting and IL5-activated blood eosinophils: two-dimensional liquid chromatography of tryptic digests in conjunction with tandem mass spectrometry (LC-MS/MS) identified peptide sequence matches (PSMs) in the UniProt database that mapped to 6,813 genes (Wilkerson *et al*., J Proteomic Res, 2016). Additionally, nearly 5,000 unique phosphopeptides were present in a fraction isolated by immobilized metal affinity chromatography prior

to LC-MS/MS. Isobaric labelling was utilized to assess proteomic and phosphoproteomic changes that accompany acute (5 minute) activation of eosinophils with IL5. By amplifying and sequencing eosinophil cDNA and using bioinformatics tools, we found that two proteoforms of NHSL2 are present in eosinophils. The sequences of these proteoforms, designated NHSL2α (1,141 residues) and β (937 residues), were longer that the 702-residue UniProt annotation and appended to the UniProt list used to re-interrogate the proteomic datasets. We used commercial antibody for immunoblotting of eosinophil lysates and immunostaining of NHSL2 in fixed and cytospun eosinophils. The cells were visualized using a confocal microscope.

Results: Using a combination of data from proteomics, transcriptomics and sequencing; we validated the presence of NHSL2α and β in eosinophils. Interrogating the proteomic dataset against the full-length sequences revealed further PSMs and seven significantly altered NHSL2 phosphosites in IL5-activated eosinophils. Commercial mouse polyclonal anti-human NHSL2 antibodies revealed a striking change in NHSL2 coinciding with the formation of nucleopods upon IL5-activation. NHSL2 staining was bright in the nucleopod tip of activated cells but difficult to appreciate in the absence of activation. Immunoblotting of eosinophil lysates indicated that the antibodies recognized a band of 150-kD in activated cells: the detected band in IL5-treated cells was far more intense despite an activation period of only 10 minutes. This unexpected immunoblotting result was in accordance with immunofluorescence in which NHSL2 staining was much brighter in IL5-treated eosinophils.

Conclusions: These investigations illustrate the challenges and rewards of buttressing proteomic data with other information, including the use of commercial antibodies made "on spec" to poorly-characterized proteins. We suspect that the commercial antibodies are sensitive to phosphorylation. We conclude tentatively that NHSL2α is heavily phosphorylated at multiple sites by kinases downstream of engaged IL5-receptor, and localizes to the nucleopod tip of polarized eosinophils. NHLS2 is not widely-expressed, and may mediate eosinophil-specific activities such as nucleopod formation.

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#7 REGULATION OF EOSINOPHIL GRANULARITY BY Myb

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Background: Organism-wide knockout of the transcription factor Myb in mice results in embryonic lethality at E15 due a severe defect in fetal liver hematopoiesis. Conditional deletion and mutation of *Myb* in adult mice has revealed its important roles in erythroid and lymphocyte lineage specification. Hypomorphic Myb mice generated by our lab and others have reportedly reduced numbers of eosinophils in the peripheral blood. Here, we have further characterized the role of Myb in eosinophil development in Myb-mutant mice.

Method: We have used genetic, molecular and cell biology approaches to examine eosinophil development in $Myb^{Plt4/Plt4}$ -hypomorphic mice, and mice in which Myb is conditionally deleted in the eosinophil lineage ($Myb^{fl/fl}$ Epx^{cre}). Differential blood analyses and flow cytometry were to quantify eosinophils, and cell complexity (granularity) was assessed by flow cytometry side scatter (SSC) Further characterization of granularity was performed with transmission electron microscopy (TEM). RNAseq was then used to explore possible transcriptional mechanisms that underpin the observed alterations in granularity. Finally, mice were challenged with intranasal papain administration to assess eosinophil migratory responses.

Results: The $Myb^{Plt4/Plt4}$ hypomorphic mutation results in reduced side scatter (a measure of cell complexity) of eosinophils isolated from diverse hematopoietic organs, as measured by flow cytometry. These alterations, and the published reduction in eosinophil numbers, are recapitulated in $Myb^{fl/fl}$ Epx^{cre} mice. Thus Myb-regulation of eosinophil number and SSC is cell-autonomous. TEM analyses suggest that the SSC alterations primarily correspond to decreased eosinophil crystalloid/specific granule content. Moreover, Myb-mutant eosinophils have decreased expression of genes encoding major granule components, at least some of which are likely direct Myb-transcriptional targets. Myb-hypomorphic eosinophils are effectively recruited to the lungs of mice following aeroallergen (papain) challenge, thus the sensory and migratory capacity of these eosinophils is not impaired.

Conclusion: In addition to its essential role in regulating embryonic fetal hematopoiesis, Myb regulates adult eosinophil number and granularity in a cell-autonomous manner.

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#8 MOUSE EOSINOPHIL RESPONSES TO LIPOPOLYSACCHARIDE (LPS) STIMULATION

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Background: Lipopolysaccharide (LPS), the major constituent of the outer membrane of gram-negative bacteria, leads to activation of human eosinophils. Human eosinophils stimulated with LPS can release cytokine and chemokines under a mechanism dependent of monocyte-derived cytokine. However, it is not clear if eosinophils from mice are able to activate and secrete in response to direct stimulation with LPS. Here, we provide a detailed investigation of the ultrastructure of murine eosinophils stimulated with LPS in order to detect morphological signs of activation and secretion. In parallel, the secretion of eosinophil-associated RNases (EARs) by these cells was evaluated.

Methods: Eosinophils isolated from the spleen of IL-5 transgenic mice were stimulated with LPS (100 ng/mL), granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/mL) or medium alone for 1 h and prepared for conventional transmission electron microscopy (TEM) and an assay to detect RNase activity of secreted mouse EARs. Quantitative TEM using the software *ImageJ* was applied to evaluate the number of intact and emptying secretory granules and vesicles associated with secretion.

Results: Mouse eosinophils stimulated with LPS exhibited clear signs of cell activation characterized by shape changes (uropods) and morphological features of PMD (enlargement, reduced electron-density, core disarrangement and/or coarse granule matrix) indicative of granule-content emptying process. The number of emptying granules found in the LPS group (30%) was similar to that induced by the physiological stimulation with GM-CSF (27%) compared to just 9% in unstimulated cells. Both LPS and GM-CSF-stimulated eosinophils significantly released mEARs compared to non-stimulated cells (20-fold). Vesiculation was a consistent event found in the cytoplasm of activated groups. These vesicles (20-150 nm in diameter) were observed in contact with secretory granules, distributed in the cytoplasm and/or beneath the plasma membrane. Quantitative TEM revealed a robust increase of the vesicle numbers in LPS-stimulated eosinophils compared to the GM-CSF and unstimulated groups (47.4 ± 5.9; 16.3 ± 3.9 and 11.4 ± 2.3 vesicles/cell section for LPS, GM-CSF and unstimulated groups respectively, mean ± SEM, n= 1193 vesicles counted in 44 cell sections). Remarkably, release of extracellular vesicles (exosomes) was also detected in the LPS-stimulated eosinophils.

Conclusions: Our data demonstrate that mouse eosinophils respond to LPS stimulation with morphological changes indicative of activation and vesicle-mediated degranulation and release of ribonucleases and exosomes. The mechanism of mouse activation by LPS waits further investigation.

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#9 BENRALIZUMAB IN HYPEREOSINOPHILIC SYNDROMES: PREDICTORS OF RESPONSE

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Background: Benralizumab is an afucosylated antibody that depletes eosinophils via antibody dependent cell-mediated cytotoxicity (ADCC). Although shown to induce rapid eosinophil depletion in asthma, the safety and efficacy in hypereosinophilic syndrome (HES), a rare and heterogeneous disorder characterized by peripheral eosinophilia >1500/mm^3 and end organ involvement, is unknown. To begin to explore potential predictors of response in HES, NK activation and killing was assessed *in vitro* in the context of a placebo-controlled, double-blind clinical trial of benralizumab in HES.

Methods: Twenty subjects with HES and AEC>1000/mm^3 on stable background therapy were randomized to receive benralizumab or placebo monthly for 3 months followed by open label benralizumab therapy. Although the study data remains blinded through week 12 until the final subject completes the placebo-controlled phase, AEC at week 13 is used as a proxy for response. To assess susceptibility to benralizumab *in vitro*, purified eosinophils (EO) and autologous NK cells (NK:EO=5) were incubated +/- benralizumab (afucosylated anti- IL5Rα) or isotype controls at 10 mg/ml. Cell death via ADCC was measured at 4h by Annexin V-7AAD staining. To evaluate the impact of the blood NK:EO ratio on benralizumab efficacy, EO depletion was also measured using peripheral blood leukocyte (PBL) after antibody incubation for 4hr. IL-5Rα expression was quantified on EO by whole blood flow cytometry.

Results: Two subjects with JAK2 mutations showed no reduction in AEC at week 13 and three additional subjects with lymphocytic variant HES (L-HES) with aberrant CD3-CD4+ T cell populations relapsed between weeks 24 and 32. The % increase in AnnexinV$^+$ EO following benralizumab compared to media ranged from 0% to 88% and was ≥10% in 10 of the 14 subjects with evaluable data in the ADCC killing assay. Of note, all 3 subjects with lymphocytic variant HES and CD3-CD4+ T cell populations who relapsed showed

a <10% increase in the *in vitro* assay; whereas the single non-responder tested had a robust *in vitro* response (36% increase). EO depletion ≥10% as compared to media was observed with benralizumab in 5 of the 20 subjects, including 4 of those who showed ≥10% killing in the ADCC assay. Although the NK:EO ratio in peripheral blood varied considerably (from 0.02 to 0.2; n=20), no relationship between NK:EO and depletion in the PBL assay was observed. Since surface expression of IL5Rα is downregulated in the setting of eosinophilia, IL5-Rα was examined on the surface of EO prior to study drug. Geometric mean (GM) expression (expressed as antibody binding capacity (ABC)) was 2083 (range 575-4265; n=16). Three subjects, including two responders and one non-responder, had ABC <1200 (2 SD below the mean of 31 normal volunteers).

Conclusions: Neither the results of the 4hr *in vitro* NK killing assay using purified cells nor those of the PBL depletion assay discriminated between benralizumab responders and non-responders, as defined by AEC response at week 13. Moreover, eosinophil expression of IL5Rα was insufficient to predict response. Additional studies examining potential predictors of response, including analysis of eosinophil and NK activation, CD16 polymorphisms and measurement of serum levels of soluble IL5Rα and IL5, are ongoing.

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#10 COMPARISON OF EOSINOPHIL ADHESION AND MIGRATION SUPPORTED BY TGF-BETA-INDUCED PROTEIN (TGFBI) AND PERIOSTIN SPLICE VARIANTS

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Background: We have previously shown that the extracellular matrix (ECM) protein periostin, which is upregulated by interleukin (IL)-13 in bronchial epithelial cells in asthma, supports alphaMbeta2 integrin (CD11b/CD18)-mediated adhesion and migration of IL-5-stimulated eosinophils. Transforming growth factor (TGF)-beta-induced protein (TGFBI) is a paralog of periostin and, like periostin, contains an amino-terminal cysteine-rich sequence and four fasciclin-1 modules. However, unlike periostin, TGFBI lacks an alternatively spliced carboxy (C)-terminal tail. TGFBI is a widely expressed ECM protein induced by TGF-beta in several cell types, including fibroblasts. We asked whether TGFBI supports eosinophil adhesion and migration and compared it to periostin.

Methods: Recombinant human TGFBI and the longest periostin splice variant (PN-L, UniProt identifier No. Q15063-1) expressed by mouse NSO myeloma cells were purchased from R&D Systems. TGFBI and the shortest periostin, lacking C-terminal sequences encoded by exons 17-19 and 21 (PN-S, UniProt No. Q15063-7) were produced in our laboratory using baculovirus in insect cells. Microtiter wells were coated with various concentrations of TGFBI or PN and blocked with fetal bovine serum (FBS). Control wells were coated with only FBS. Purified human blood eosinophils were added to wells in the presence or absence of IL-5, IL-3, or granulocyte macrophage-colony stimulating factor (GM-CSF). Adhesion was quantified after 1 h, using an eosinophil peroxidase assay. Random motility was studied with a microbead monolayer assay; after 20 h, wells were viewed in an inverted microscope, photographed, and migratory tracks were quantified using Fiji software. Differences with $p \leq 0.05$ (ANOVA with Dunnett's or Tukey's post tests, or t test) were considered significant.

Results: Cytokine-stimulated eosinophils adhered to TGFBI in a concentration-dependent manner. This concentration dependence was similar to that of PN-L or PN-S. Adhesion to TGFBI was blocked by anti-alphaM or beta2 integrin antibodies and was stimulated to a higher degree and at a lower concentration by GM-CSF than by IL-5 or IL-3. TGFBI supported cytokine-stimulated eosinophil migration but was less effective than PN-L or PN-S (in the presence of IL-5, 10 ng/ml). Further, PN-S was less effective in supporting migration than PN-L. Finally, IL-3 stimulated migration on TGFBI or PN-L to a greater degree than did IL-5 or GM-CSF.

Conclusions: TGFBI supported alphaMbeta2 integrin-mediated adhesion and migration of cytokine-stimulated eosinophils, indicating that TGFBI and periostin support adhesion by similar mechanisms. Quantitative differences in migration on TGFBI, PN-L, and PN-S indicate that alternatively spliced sequences in the C-terminal tail of periostin are important for maximal eosinophil migration. GM-CSF is the optimal cytokine for adhesion at 1 h, whereas IL-3 is the optimal cytokine for migration during 20 h. In conclusion, TGFBI is a novel eosinophil ligand that may be important for eosinophil recruitment and retention *in vivo* in TGF-beta-rich tissues. In addition, the IL-5 family cytokines appear to have different efficacies in eosinophil adhesion and migration, and may be differentially important at various times after eosinophil activation.

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#11 PI3K/MAPK BLOCKADE INHIBITS EOSINOPHIL EXTRACELLULAR TRAP CELL DEATH

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Background: Eosinophil extracellular trap cell death (EETosis) is an active form of cell death that mediates lytic degranulation. Immobilized immunoglobulins are potent EETosis inducers in a NADPH oxidase-dependent manner. Phosphoinositide 3-kinases (PI3Ks) and MAP kinases (MAPKs) have been known to regulate fundamental eosinophil responses, although their involvement in EETosis has not been studied.

Methods: We utilized an *in vitro* system using purified human blood eosinophils. After incubation with PI3K inhibitors (PI3Kγ selective inhibitor AS605240, pan-PI3K inhibitor LY294002) or MAPK inhibitors (ERK1/2 inhibitors U0126 and PD98059, p38 inhibitor SB202190), eosinophils were stimulated with immobilized IgG and IgA for 3 h. Cell death, reactive oxygen species (ROS) production, and release of filamentous chromatin structure (DNA traps) were assessed using SYTOX intensity, APF assay, and immunostaining for extracellular histone H1, respectively.

Results: Immobilized immunoglobulin-induced cell death, ROS production, and DNA trap production were almost completely inhibited by LY294002 and all MAPK inhibitors we tested, and partially by AS605240. After stimulation, LY294002 and MAPK inhibitor-treated cells indicated bi-lobed nuclei, although round nuclear shape change was observed in the majority of AS605240-treated cells.

Conclusions: The current results indicate that the PI3K/MAPK-NADPH oxidase activation process plays an important role in immobilized immunoglobulin-induced nuclear shape change and EETosis.

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#12 FOR WHOM THE EOS TOLL: EFFECT OF MICROENVIRONMENT ON MURINE EOSINOPHIL TOLL-LIKE RECEPTOR EXPRESSION

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Background: Toll-like receptors, while commonly expressed on antigen presenting cells, are also found on both human and mouse eosinophils (Kvarnhammar and Cardell 2012). As residents of the gut mucosa at homeostasis, and migrants to the lung in response to allergen challenge, eosinophils are exposed to a diverse array of microflora that may influence their antimicrobial activity. We sought to investigate the expression and plasticity of Toll-like receptors in murine eosinophils, focusing on variations between organs and in response to microbial and TLR agonist stimulation.

Methods: Eosinophils were isolated from lungs and spleen of interleukin-5 transgenic (IL5tg) mice and evaluated at baseline and in response to varying concentrations of LPS for 1-24 hours. Bone marrow eosinophils (bmEos) were generated from wild-type mice as per standard methods. Cell surface and intracellular TLR expression was determined by flow cytometry using PE-conjugated anti-mouse TLR2 clone CB225, AF488-conjugated anti-mouse TL4 clone UT41, and PE-conjugated anti-mouse TLR7 clone A94B10.

Results: Eosinophils from IL5tg mice were found to express TLR4, but not TLR2, and, surprisingly, not TLR7. Interestingly, expression of TLR4 by eosinophils varied between organs, with a larger proportion of lung eosinophils (12 ± 4%) expressing TLR4 than spleen eosinophils (4 ± 3 %) or those cultured from bone marrow (0%). Furthermore, eosinophils from the bone marrow all stained 100% positive for intracellular TLR4. Isolated lung eosinophils showed an increase in TLR4 median fluorescence index (MFI) after *ex vivo* culture with increasing concentrations of LPS, while TLR4 MFI for spleen eosinophils remained constant in response to LPS stimulation. We will continue to explore TLR4 expression in response to challenge both *ex vivo* and *in vivo*, including responses to the gram-negative microorganism *Haemophilus influenzae*, and likewise the presence of TLR4 on eosinophils from the gastrointestinal tract. We will also investigate cytokine release in response to LPS challenge, focusing on mediators including IL-10, IL-4, and interferon gamma.

Conclusions: TLR4 expression on murine eosinophils has been shown to vary between organs, indicating a plasticity in eosinophil phenotype that may be influenced by exposure to different microenvironments.

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#15 EOSINOPHILS IN A THREE-DIMENSIONAL COLLAGEN GEL *IN VITRO* HAVE DECREASED SURFACE L-SELECTIN (CD62L)

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Background: We and others have previously studied the activation state and morphology of blood and bronchoalveolar lavage (BAL) eosinophils, as well as the activation of suspended eosinophils by various factors *in vitro*. Further, most adhesion and migration studies have been made with eosinophils adhering to a two-dimensional layer of extracellular matrix (ECM) or other proteins coated on plastic. However, the activation state of eosinophils in a three-dimensional (3D) matrix has not been extensively studied. We are establishing an *in vitro* model consisting of a collagen gel to study eosinophils in a 3D matrix environment.

Methods: Purified human blood eosinophils were mixed with collagen type I and RPMI medium and added to wells, and the gel was allowed to polymerize embedding eosinophils. After polymerization for 1 h, recombinant collagenase G in RPMI was added to digest the gel. Eosinophils recovered after digestion were analyzed by flow cytometry for viability using the fixable dye Fluor660 and for cell-surface expression and activation state of potential biomarkers of eosinophil activation, including integrins, cytokine receptors, and L-selectin (CD62L). Control cells were treated identically except did not receive collagen, i.e., were not embedded in a gel but were incubated with RMPI and treated with collagenase G.

Results: Eosinophils embedded in a 3D collagen I gel and recovered with collagenase G as well as control cells exposed to collagenase G were 96-97% viable. Eosinophils recovered from a gel were found to have 19% ± 3 % (mean ± SEM, as specific median channel fluorescence) or 39% ± 5% (as specific median fluorescence intensity) lower surface expression of L-selectin (CD62L) than did collagenase-treated cells not in collagen ($p = 0.02$ and 0.01, respectively, paired t test). Levels of integrins (beta1 [CD29], beta2 [CD18], and alphaM [CD11b]), activation state of beta1, or levels of cytokine receptors (interleukin [IL]-5, IL-3, granulocyte macrophage-colony stimulating factor [GM-CSF] receptor alpha subunits [CD125, CD123, and CD116], and common beta subunit [CD131]) were not significantly different (≤ 14% or 23%, respectively) between gel-recovered and control cells.

Conclusions: Eosinophils embedded in a 3D matrix *in vitro* have significantly lower surface L-selectin (CD62L) than eosinophils in suspension. Thus, 3D matrix-embedded eosinophils may acquire a partly different phenotype or activation state. We aim to use this *in vitro* 3D matrix model to also study cell morphology, subcellular protein localization, adhesive structures, and activation of signaling proteins of gel-embedded eosinophils, and compare to cells cytospun from suspension or cells adherent to a 2D protein layer, as well as to study potential cell migration in, into, or out of the gel and compare to migration in a 2D system. Experience and results from studying eosinophils in a 3D matrix *in vitro* may in the future aid the study and understanding of eosinophils in tissue samples, e.g., from lung tissue in asthma.

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#16 MANIPULATION OF MCL-1 CRITICALLY ALTERS MOUSE BONE MARROW-DERIVED EOSINOPHIL APOPTOSIS.

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Background: Due to the relatively short life span and inability of isolated human eosinophils to proliferate it is particularly hard to genetically manipulate these cells using techniques such as siRNA or viral transfection. Generation of bone marrow-derived eosinophils (bmEos) allows experimentation on mature, morphologically and phenotypically distinct cells from mice already possessing a genotype/phenotype of interest. Previous work from our laboratory has suggested that the anti-apoptotic Bcl-2 member Mcl-1 is an important regulator of eosinophil viability, with pharmacological down regulation of Mcl-1 occurring concurrently with apoptosis. Culture of bmEos allows further investigation of the role of Mcl-1 in eosinophil apoptosis.

Methods: Human eosinophils were isolated from peripheral blood via negative magnetic selection following centrifugation through discontinuous Percoll gradient. Mouse bone marrow was isolated and bmEos were generated over 14 days in the presence of 100ng/mL stem cell factor and 100ng/mL FLT3 ligand from days 0-4, followed by 10ng/mL recombinant mouse IL-5 for the remaining 10 days. Human and mouse eosinophils were cultured over 48-hours with/without pharmacological inducers of eosinophil apoptosis (the cyclin-dependent kinase inhibitor AT7519 and the flavone wogonin). Viability and apoptosis of bmEos from human transgenic Mcl-1 (hMcl-1) and wild-type (WT) mice were assessed by flow cytometry (Annexin V / propidium iodide (PI) staining) and cytocentrifuge preparations.

Results: Human eosinophils and mouse bmEos underwent time-dependent constitutive apoptosis. AT7519 and wogonin induced time- and concentration-dependent apoptosis of human eosinophils and mouse bmEos. Eosinophil apoptosis was preceded by down-regulation of intracellular Mcl-1 and occurred in conjunction with cleavage of caspase-3. bmEos from hMcl-1 mice were sig-

nificantly (>25%) more viable over 48 hours compared to WT bmEos (WT; 22.4% ± 4.9, hMcl-1; 47.9% ± 7.2, *p<0.05; n=4-5, ± SEM).

Conclusions: These data provide further evidence for the potential of influencing eosinophils apoptosis by pharmacological or genetic manipulation of Mcl1 levels, and demonstrate that Mcl-1 plays an important role in regulating eosinophil apoptosis. Importantly, isolation and culture of bmEos from wild type and knockout/transgenic mice provides a useful model system to investigate the role of Mcl-1 and other potential regulators of eosinophil apoptosis, and generates useful data to inform future pre-clinical *in vivo* experiments.

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#17 ADULT EOSINOPHILIC ESOPHAGITIS PATIENTS' SATISFACTION WITH DIFFERENT TREATMENT MODALITIES

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Background: Treatment options for eosinophilic esophagitis (EoE) patients include drugs (proton-pump inhibitors [PPI], swallowed topical corticosteroids [STC]), food elimination diets, and esophageal dilation. Knowledge about patients' view regarding the different therapeutic options is very limited. We aimed to assess adult EoE patients' satisfaction with different EoE-specific treatment modalities used in the past 12 months.

Methods: We first created a questionnaire that included items that queried general demographic characteristics (7), EoE-specific patient history and presence of atopic disease (8), past and present EoE-specific therapy (9), concomitant medication use (7), considerations for therapy choice (2), and satisfaction with various therapies recalled over a period of 12 months (assessed using the validated "Treatment Satisfaction Questionnaire for Medication" [TSQM], 52). The TSQM consists of 14 items falling into 4 scales: effectiveness (3), side effects (5), convenience (3), and overall satisfaction (3). The score for each scale ranges from 0 (dissatisfied) to 100 (satisfied). Three psychologist-guided focus groups with EoE patients were conducted to inform the content and the structure of the questionnaire and ensure that patient understand the items, instructions, and response options. The questionnaire was sent to 148 patients in Switzerland.

Results: Patient response rate was 74% (108/147). Mean patient age was 46.3 years (SD = 15.9), 85/108 patients (79%) were male, and mean disease duration was 6.8 years (SD = 5.1). In the last 12 months, 25%, 84%, 19%, and 13% were treated with PPI, STC, food elimination diet, and esophageal dilation, respectively (37.0% patients received more than one treatment; 7.4% of patients did not receive any treatment). Patients identified the following considerations as important for the therapy choice: the treatment effect on the symptoms (89%), the treatment effect on esophageal inflammation (76%), possible side effects (69%), ease of therapy use (58%), physician's recommendation (50%), and compatibility of therapy with lifestyle (46%). When asked about the single most important criterion for the choice of therapy, 49%, 34%, and 12% of patients chose the effect of treatment on symptoms AND esophageal inflammation, the effect of the treatment on the symptoms, and the effect of treatment on esophageal inflammation, respectively, as deciding factor. The TSQM scales scores as well as average TSQM values for patients on PPI, STC, and diet are shown in Table 1.

Conclusions: Adult EoE patients consider both effect of medication on symptoms and esophageal inflammation as important criteria, when choosing EoE therapy. EoE patients appear to be satisfied with PPI, STC, and dietary therapy.

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Table 1. Median TSQM scores and IQR. *For a side-effect scale, a score of 100 is given to patients, who do not experience side effects.

TSQM scales	PPI (n = 27); median treatment duration 6 years [3 - 9]	STC (n = 84; median treatment duration 5 years [2 - 6]	Diet (n=21; median treatment duration 2 years [1 – 4.5]
Effectiveness	66.7 [38.9-77.8]	83.3 [66.7 – 94.4]	77.8 [50 – 88.9]
Side-effects*	100 [100 – 100]	100 [100 – 100]	100 [100 – 100]
Convenience	88.9 [77.8 – 100]	83.3 [66.7 – 100]	50 [33.3 – 66.7]
Overall satisfaction	71.4 [50 -85.7]	78.6 [64.3 – 92.9]	78.6 [57.1 – 92.9]
Average score	79.8 [69.4 – 85.5]	84.4 [72.8 – 92.3]	76.6 [59.8 – 81.9]

#18 CLINICAL PICTURE AND BLOOD EOSINOPHIL PHENOTYPE OF CHILDREN WITH COLLAGENOUS GASTRITIS

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Background: Collagenous gastritis (CG) is a rare disorder in children with only a limited number of case reports. It is histologically characterized by subepithelial deposits of collagen bands and dense infiltrates of eosinophils and inflammatory mononuclear cells in the gastric mucosa. Endoscopically, the typical finding is a thickened and nodular mucosa in the gastric body. The underlying pathogenesis of the disorder is unknown. The objectives of this study were: 1) to define the clinical picture of pediatric CG; and 2) to investigate if blood eosinophils display a molecular pattern in children with CG that differs from that of healthy children.

Methods: Blood eosinophils from eight children (median age 13 y, range 10-17 y) with CG and age-matched healthy controls were analyzed with flow cytometry regarding levels of CD23, CD44, CD54, CRTH2, FOXP3 and galectin-10.

Results: The patients presented with iron deficiency anemia and about half of them had recurrent abdominal pain. Notably, all children with CG were girls. None of the patients had any concomittant allergic or autoimmune disease or blood eosinophilia. The collagenous inflammation affected the ventricle only and was not detected in the small bowel or colon in any of the patients. Children with CG had a blood eosinophil phenotype characterized by significantly higher levels of CD44 on their surface and increased intracellular levels of the transcription factor FOXP3 and of the immunoregulatory protein galectin-10 compared with healthy controls.

Conclusions: Despite the dense eosinophil infiltration of the gastric mucosa, pediatric CG does not seem to be associated with allergic disease. The eosinophils in the circulation of children with CG display a molecular pattern that differs from that of healthy children.

#19 DOES THE GRADE OF T-CELL INFILTRATION CORRELATE WITH EOSINOPHIL COUNTS OR SYMPTOMS IN EOSINOPHILIC ESOPHAGITIS?

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Background: Eosinophil infiltration in the esophageal mucosa in combination with symptoms of esophageal dysfunction is fundamental for the diagnosis of eosinophilic esophagitis. A correlation between the two would therefore be plausible. However, in an earlier study we have not been able to discover any such correlation. Eosinophilic esophagitis is claimed to be a T-cell mediated disease. Consequently, the purpose of the present study was to search for a correlation between symptoms and T-cell counts in CD4 and CD8 antibody stained slides.

Methods: The study included 64 patients with active EoE diagnosed at the ENT department at NÄL Hospital, Trollhättan. Histological samples gathered from the proximal and distal esophageal mucosa were stained using both hematoxylin and eosin (HE) and an immunohistochemical (IHC) technique against "Eosinophil Major Basic Protein," visualizing the eosinophilic cells and against CD4 and CD8 visualizing the T-cells.

The peak number of cells per high power field (HPF =0.22mm^2;range 0.21-0.24) was assessed. Symptoms and Health Related Quality of life data were recorded using the Watson Dysphagia Scale (WDS) and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire–Oesophageal Module 18 (EORTC QLQ-OES18). The WDS scale measures dysphagia and has been used in several studies of patients with esophageal dysphagia. The EORTC QLQ-OES18 evaluates dysphagia and eating problems.

Results: Data from 64 patients with active EoE were available except for 5 patients with missing or incomplete data in the WDS. The mean age was 46 years and 73% were men. The number (Mean;SD) of eosinophils per HPF in HE and EMBP-stain, and T-cells in CD8 and CD4 stain was (34.3;18.4), (69.8; 54.0), (122.6;65.0) and (12.8;10.8) respectively. No substantial correlation was found between the number of CD4 and CD8 cells, nor between eosinophil and T-cell counts by any staining method. The mean value of CD8 cells was 10 times higher than CD4 cells. Finally, no correlation was found between T4 or T8 cell count and any of the 3 symptom scores.

Conclusions: A T-cell infiltration with predominance of CD8 cells was found in the esophageal mucosa of patients with active EoE but no correlation to the number of eosinophils or to symptom severity was found. The immunopathology of eosinophilic esophagitis remains unclear.

#20 INITIAL CLINICAL PRESENTATIONS OF PATIENTS WITH HYPEREOSINOPHILIC SYNDROME AT A TERTIARY MEDICAL CENTER

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Background: Patients diagnosed with hypereosinophilic syndrome (HES) are often diagnosed after presentation with severe organ damage resulting from eosinophilia such as pulmonary, cardiac or embolic disease. The goal of this study is to determine the presenting symptom in patients diagnosed with HES.

Methods: A retrospective review of electronic medical records (January 2010 to December of 2012) was performed. The criteria for inclusion of patients in the study followed the WHO criteria for the diagnosis of HES. The search diagnoses included eosinophilia, hypereosinophilia and hypereosinophilic syndrome. Patients with eosinophilic granulomatosis with polyangiitis, other vasculitides and clonal hematologic disorders were excluded. Among 150 identified patients, 12 were found to fulfill the diagnostic criteria of HES. Those underwent detailed chart review. The study was approved by the institutional review board.

Results: Of the 12 patients (age range 20-76; 58.3% male) with HES, 5 were initially seen by Hematology, 3 by Cardiology, 2 by Allergy and 1 each by Gastroenterology and the Medical ICU. The most common presenting signs or symptoms were pulmonary (66.7%), cardiovascular (58.3%), gastrointestinal (50%), and constitutional (e.g. fever and fatigue; 50%) in nature. Dermatologic (25%), musculoskeletal (25%), and hematologic (8.3%) signs and symptoms were also noted. Dyspnea (50%) was the single most commonly reported initial sign or symptom prior to HES diagnosis followed by fatigue (25%), fever (25%), nausea (25%), and peripheral edema (25%). Other symptoms included episodes of hypotension (16.7%), cough (16.7%), chest pain (16.7%), diarrhea (16.7%), myalgia (16.7%) and arthralgia (8.3%), abdominal pain (8.3%), lymphadenopathy (8.3%), angioedema (8.3%) and urticaria (8.3%). The average symptom duration was 2.8 years but ranged from 1 month to 13 years. Eosinophil counts at the time of symptom onset ranged from 2,370 to 29,394 eosinophils per microliter. Four patients had end-organ manifestation of cardiovascular disease at the time of initial evaluation and were 15 years older on average compared to the entire cohort. Coronary artery disease manifested in two patients at 4 and 30 years before the development of signs and symptoms that led to referral. In the two other patients, pulmonary hypertension and congestive heart failure developed concurrent to the finding of eosinophilia. All patients had detailed work-up to rule out other causes of eosinophilia. Initial bone marrow biopsies showed eosinophilia in all patients without evidence for clonal hematologic disorder.

Conclusions: The initial signs and symptoms of HES are often non-specific and can lead to referral to medical specialists outside of Allergy and Hematology. Several patients had evidence of end-organ disease at the time of referral. Early recognition and diagnosis of HES is essential to minimize morbidity. Patients with eosinophil counts above 1,500 eosinophils per microliter should be evaluated by an allergist or hematologist to rule out secondary causes and initiate appropriate treatment.

Grant Support: Mayo Foundation.

#21 EVALUATION OF EOSINOPHIL BIOMARKERS IN EOSINOPHILIC ESOPHAGITIS PATIENTS

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Background: Monitoring disease activity in eosinophilic esophagitis is challenging as there is a lack of validated tools to replace invasive procedure-based endoscopy and pathology evaluation. We aim to identify a profile of eosinophil surface biomarkers that will correlate with eosinophil activation and disease activity in eosinophilic esophagitis. Previous studies have demonstrated that expression of eotaxin in esophageal tissue is a prominent mediator for the recruitment and activation of eosinophils in eosinophilic esophagitis. Several eosinophil surface proteins such as CD11c, CD18, CD23, CD44, CRTH2, CCR3 have been shown to be up- or down-regulated in patients with EoE. The intermediate activity state of beta1 integrin on eosinophils, reported by monoclonal antibody N29, has been associated with impaired pulmonary function in non-severe allergic asthma. Furthermore, the intermediate activity state of beta2 integrin on eosinophils has been associated with airway eosinophilia in asthmatic patients.

Methods: Peripheral blood eosinophils purified by density centrifugation and negative selection using AutoMACS were stimulated with eotaxin. In an effort to identify biomarkers that correlate with EoE disease activity, previously identified eosinophil surface proteins associated with activation or allergic diseases were selected for analysis by flow cytometry. This pilot experiment was designed to concurrently identify biomarkers including: alphaL, alphaM, beta1, and beta2 integrins, activated beta1 integrin moni-

tored by mAb N29, CCR3, CD40, CD44, CD66b, CRTH2 and P-selectin glycoprotein ligand-1 (PSGL-1), as well as alphaIIb integrin (marker of associated platelets), and cell-surface-associated P-selectin

Results: Eotaxin stimulation of purified eosinophils upregulates surface protein expression of beta2, alphaM and alphaL integrins, and CD66b; while downregulating CRTH2, CCR3, and N29 signals. No major changes in the expression of the other biomarkers were observed.

Conclusions: Eotaxin stimulation of eosinophils results in the upregulation or downregulation of several surface biomarkers. We expect that the modulation of the surface expression of these biomarkers will be observed and will correspond to changes in eosinophilic esophagitis disease activity.

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#22 EOSINOPHILIC GASTROENTERITIS ASSOCIATED WITH HIRSCHSPRUNG'S DISEASE AND ITS ALLIED DISORDERS

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Background: Eosinophilic gastroenteritis (EGE) sometimes occurs secondary to non-eosinophilic digestive disorders. Only a few reports about EGE associated with Hirschsprung's disease (HD) and its allied disorders (HAD) are available. We employed treatments aimed at reversing EGE for HD/HAD patients with EGE in our hospital and reviewed their clinical courses.

Methods: Three HD/HAD patients with EGE, who presented to our hospital between 2012 and 2016, were included. The diagnosis was based on gastrointestinal symptoms and eosinophilic infiltration of the gastrointestinal mucosa with ≥20 eosinophils/high-power field (eo/HPF) in the presence of HD/HAD and the patients received treatments aimed at reversing EGE. Clinical data, including imaging, histological findings, and laboratory examinations were retrospectively reviewed.

Results: Two patients had HAD and one patient had HD. All patients had short bowel syndromes due to bowel resection. Clearly identified causative foods were Hen's egg for all patients and cow's milk for one patient. Results of immunoglobulin E antibody tests for egg white were positive in all patients while those for cow's milk and wheat were positive in one patient each. The peak gastrointestinal eosinophil counts ranged from 50 to 250 eo/HPF. Two patients with HAD were successfully treated by eliminating causative foods and a modified empiric six most common food elimination diet, respectively. One patient with HD required systemic prednisolone and became dependent.

Conclusion: We speculate that intestinal dysfunction and/or short bowel could negatively affect the tolerance of foods. These cases might provide a clue as to the underlying cause of EGE.

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#23 EOSINOPHILIC ESOPHAGITIS RISK VARIANT AT 2P23 DAMPENS IL-13-INDUCED CALPAIN-14 PROMOTER ACTIVITY IN A STAT6-DEPENDENT MANNER

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Rationale: Eosinophilic esophagitis (EoE) is a chronic, food-driven, esophageal, inflammatory allergic disease. We have recently found that, in addition to genetic risk loci for allergic sensitization, EoE susceptibility is linked to a tissue-specific genetic factor(s) at 2p23, encoding the CAPN14 gene. In our initial studies we showed that CAPN14 is dynamically up-regulated as a function of EoE disease activity and after exposure of epithelial cells to IL-13, a critical regulator of esophageal inflammation in EoE. Patients with EoE and the 2p23 risk haplotype express decreased esophageal CAPN14.

Methods: We performed a replication and fine-mapping study of the 2p23 locus in an additional cohort of subjects with and without EoE. Data from the GTEx database was used to confirm the specificity of CAPN14 expression and establish genotype-dependent expression in subjects without EoE. We used DNA affinity precipitation analysis and electromobility shift assays to identify proteins that differentially bound specific variants. The regulation of CAPN14 expression was compared to CCL26 in the context of epithelial cells confluence, calcium concentration, air liquid interface differentiation, and IL-13 exposure. Luciferase reporter assays were used to further define the IL-13 and genotype-dependent parts of the CAPN14 promoter.

Results: Using an independent genetic cohort, we replicated the 2p23 EoE-risk locus (rs76562819 $p_{meta}<10^{-18}$, Odds Ratio=1.98) and identified five genetic variants most likely to be causal. CAPN14 expression is specific to the esophageal mucosa as compared with other tissue types including the esophageal muscularis and the esophageal gastric junction. In esophageal mucosal samples from a control cohort, CAPN14 was expressed as a function of rs76562819, and control subjects with the EoE-risk genotype expressed less CAPN14 than those with the non-risk genotype ($p<10^{-8}$). We identified specific differentiation factors that are necessary for IL-13 induced CAPN14 in the two esophageal epithelial cell lines EPC2 and TE7. Using luciferase reporters transfected into esophageal epithelial cells, we identified the critical promoter elements of CAPN14 using promoter deletion constructs. We demonstrated STAT6 binding to the three putative binding sites in the promoter and first intron. Each of the three STAT6 elements were required for the 10-fold increase in IL-13 induced promoter activity and for the 50% reduction in genotype-dependent expression.

Conclusions: Our work establishes a candidate molecular mechanism for EoE disease etiology in which the risk variant rs76562819 at 2p23 dampens IL-13-induced CAPN14 promoter activity in a STAT6-dependent manner in differentiated esophageal epithelia.

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#24 DOWN-REGULATED EOSINOPHIL ACTIVITY IN ULCERATIVE COLITIS WITH CONCOMITANT SEVERE CHOLANGITIS

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Background: Primary sclerosing cholangitis (PSC) is a chronic bile duct inflammation affecting every twentieth patient with ulcerative colitis (UC). The reason why a subgroup of patients with UC develops this severe condition is unclear, and the role of intestinal immune cells in the pathogenesis of PSC remains to be established. The eosinophil granulocyte is one of the immune cells implicated in the inflammatory process of UC. Our aim was to define the role of eosinophils in the colonic inflammation in PSC-UC, and to characterize the inflammatory protein profile in the same tissue.

Methods: We studied eosinophil accumulation and activation in colonic tissue, comparing UC-patients with and without concomitant PSC. The study included 22 patients with PSC + UC, 28 with UC and 19 controls. Biopsy samples from three locations of the colon were collected. Eosinophil expression of activation markers (CD66b, CD44 and CD69) was analyzed by flow cytometry; eosinophil numbers were established by immunohistochemistry (EPO). The colonic level of a range of inflammatory mediators was assessed using a multiplex proximity extension assay.

Results: Colonic eosinophils were more abundant in active UC and PSC-UC compared with controls, and eosinophil numbers correlated with protein levels of CCL11 in colonic tissue. Eosinophil expression of activation markers was significantly increased in UC only. Several inflammatory mediators, such as IL-33, IL-1α and IL-17, were increased in ulcerative colitis but not in the patients with concomitant PSC.

Conclusions: These data suggest that eosinophils are accumulated to the colonic mucosa in patients with PSC-UC, and that CCL11 contributes to the recruitment. However, low levels of inflammatory mediators in the mucosa may be an explanation to the lack of eosinophil activation in this patient group. We show for the first time that eosinophil activation phenotype discriminates between UC and PSC-UC, and that this may depend on the local protein profile of the colonic mucosa.

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#25 PULMONARY EOSINOPHILS INCREASE VAGUS NERVE MEDIATED AIRWAY REFLEX RESPONSE IN MICE

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Background: Eosinophils mediated effects on airway nerves are important in causing airway hyperresponsiveness. In these studies, we differentiated the effects of circulating eosinophils from airway eosinophils in changing airway nerve function.

Methods: We used NJ1638 mice (which express interleukin-5 in T cells, and have peripheral blood eosinophilia), NJ1726 mice (which express IL-5 in airway epithelium and have airway and peripheral blood eosinophilia), PHIL mice (which express diphtheria toxin A-chain driven by the eosinophil peroxidase promoter, and are devoid of eosinophils) and NJ1726-PHIL (generated by cross breeding NJ1726 and PHIL mice). All mice have C57BL/6 background. Changes in airway resistance in response to aerosolized serotonin (10-300 mM) before and after vagotomy were measured. M3 muscarinic receptor function on airway smooth muscle was tested by measuring changes in airway resistance in response to aerosolized methacholine (10-300mM) after vagotomy. Differential counts of inflammatory cells in bronchoalveolar lavage (BAL) and blood were performed.

Results: Blood eosinophils were increased in both NJ1638 and NJ1726 mice compared with wild-type. In contrast, only NJ1726 mice had increased eosinophils in BAL. Both PHIL and NJ1726-PHIL mice have undetectable eosinophils in blood and BAL. Without vagotomy, changes of airway resistance in response to aerosolized serotonin in NJ1726 mice were significantly increased compared to wild type. In contrast, airway resistance in response to aerosolized serotonin was not increased in NJ1638 or NJ1728-PHIL mice compared to wild type, showing that eosinophils, and not IL-5, mediate this increase in airway reflex bronchoconstriction. Vagotomy or treatment with atropine blocked serotonin induced bronchoconstriction, demonstrating that it was mediated via vagal reflex. Increases in airway resistance in response to methacholine in vagotomized mice were not different among strains of mice, indicating that the M3 muscarinic receptor function was unchanged.

Conclusions: Our data show that only pulmonary eosinophils, not circulating eosinophils or airway IL-5, increase vagally mediated reflex bronchoconstriction. Therapeutic interventions that block or reduce pulmonary eosinophils in asthmatic individuals may efficiently inhibit vagus nerve mediated airway hyperreactivity and reduce asthma symptoms.

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#26 EOSINOPHILS REGULATE AIRWAY PARASYMPATHETIC NERVE GANGLION STRUCTURE

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Background: Eosinophils interact directly with airway nerves in patients with asthma and in animal models of asthma. In this study we investigated the effects of eosinophils on airway ganglion structure and neurotransmitter expression.

Methods: Adult wild-type mice (WT, C57Bl/6), eosinophil-deficient mice (PHIL, with diphtheria toxin expression driven by the eosinophil peroxidase promoter), and mice with airway eosinophilia (NJ1726, with IL5 expression driven by the CC10 airway epithelium-specific promoter) were studied. Whole mount tracheas were labeled with an antibody against the pan-neuronal marker PGP9.5 and with antibodies against substance P or neuronal nitric oxide synthase (nNOS). Tracheas were optically cleared by dehydration in ethanol followed by rehydration with benzyl alcohol/benzyl benzoate. Nerves were imaged in three dimensions with confocal microscopy. The number of airway ganglia, and the number of neurons within ganglia expressing specific neurotransmitters, were counted by blinded observers. Data are expressed as mean±SEM and analyzed with one-way ANOVA.

Results: WT mice had 67±13 ganglia/trachea (n=45). Compared to WT, eosinophil deficient mice had significantly more airway ganglia (88±13 ganglia/trachea, n=25, $p<0.0001$), while mice with airway eosinophilia had fewer ganglia (56±14, n=20, $p<0.0001$). Differences in ganglia number were primarily due to variability in small ganglia, defined as 1-5 neurons in size (52±2 small ganglia in WT, 66±2 in eosinophil deficient, 40±3 in mice with airway eosinophilia, $p<0.0001$). Exceptionally large ganglia (greater than 250 neurons) were seen more frequently in mice with increased eosinophils (present in 15% of WT mice, 29% of mice with airway eosinophilia, 0% of eosinophil-deficient mice).

Eosinophils decreased the number of substance P-expressing neurons (5±1% in WT, 6±3% in eosinophil deficient mice, 2±2% in mice with airway eosinophilia, $p=0.02$). Similarly, the number of nNOS-expressing neurons was increased in the absence of eosinophils (19±5% in WT, 27±4% in eosinophil-deficient mice, $p=0.01$).

Conclusions: Eosinophilia affects the organization and neurotransmitter content of airway nerves, which may affect airway function.

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#27 OZONE AND SENSITIZATION ALTER TISSUE, LAVAGE AND NERVE-ASSOCIATED EOSINOPHILS IN THE LUNG

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Background: Airway hyperreactivity 1 day after ozone exposure is eosinophil-dependent and neuronally-mediated. Paradoxically, eosinophils recruited by TNFα attenuate airway hyperreactivity 3 days after ozone in non-sensitized animals. This protective effect is lost following sensitization.

Aim: We evaluated the effects of ozone exposure, ovalbumin sensitization and TNFα on eosinophils in the airway subepithelium, along airway nerves and in bronchoalveolar lavage fluid (BAL).

Methods: Lungs from female Hartley guinea pigs exposed to ozone (2.0 ppm) or filtered air (4 hours) were harvested 3 days later. Some animals were sensitized to ovalbumin 21 days before ozone and/or treated with the TNFα antagonist etanercept 3 hours before ozone. Nerves and eosinophils were stained with PGP9.5 and chromotrope 2R, respectively. Images were obtained using an Apotome.2 confocal microscope (Zeiss, 40X, 1.4 NA) and analyzed with ImageJ. Eosinophils within 8 µm of nerves were classified as nerve-associated. The Animal Care and Use Committee approved all protocols.

Results: Small non-cartilaginous airways have ~2 fold more tissue and nerve-associated eosinophils versus large cartilaginous airways. Nerve-associated eosinophils account for a quarter of all eosinophils in small airways, whereas only a sixth of all eosinophils were associated with nerves in large airways. Ozone decreased tissue eosinophils without changing nerve-associated eosinophils, and increased BAL eosinophils in non-sensitized animals. Sensitization did not alter tissue eosinophils, but doubled nerve-associated and BAL eosinophils. In contrast to non-sensitized animals, ozone reduced nerve-associated eosinophils in sensitized animals. Etanercept increased eosinophils in small airways of non-sensitized and sensitized animals. Etanercept also completely blocked the ozone-mediated decrease in tissue eosinophils in non-sensitized animals, and partially blocked this effect in sensitized animals. Etanercept had no effect on nerve-associated eosinophils in non-sensitized animals. In contrast, etanercept decreased nerve-associated eosinophils in sensitized air control animals. This led to similar numbers of nerve-associated eosinophils in air- and ozone-exposed sensitized etanercept-treated animals.

Conclusion: Eosinophils are protective 3 days after ozone exposure and are found in abundance along airway nerves. Both sensitization and etanercept alter the role of eosinophils in ozone-induced hyperreactivity by increasing BAL eosinophils, while nerve-associated eosinophils are decreased and their beneficial effects are lost. Thus, BAL and tissue eosinophils fail to predict eosinophils' presence or effects on airway nerves.

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#28 FOCAL ADHESION KINASE (FAK) INHIBITION BLOCKS EOSINOPHIL RECRUITMENT IN RESPONSE TO IL-4 *IN VITRO* AND *IN VIVO*.

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Background: Focal adhesion kinase (FAK) is involved in cell migration and metastasis. FAK signaling also affects other aspects of the tumor microenvironment. Because of this, a number of FAK inhibitors have been developed and are in clinical trials as cancer therapeutics. We recently showed that expressing a dominant negative form of FAK in endothelial cells had the unexpected effect of blocking eosinophil recruitment to IL-4 stimulated endothelial cells. In this study, we examined the effect of FAK inhibitors on eosinophil recruitment *in vitro* and *in vivo*.

Methods: Human endothelial cells were treated with two structurally unrelated FAK inhibitors (PF-573228 and FAK-14) and then stimulated with IL-4. After 24 hours eosinophils were perfused over the endothelial cells under laminar flow conditions and rolling, adhesion and transmigration were measured. To examine this *in vivo,* mice pre-treated with PF-573228 were injected locally with IL-4 and leukocyte recruitment in the cremaster muscle was determined 24 hours later using intravital microscopy. We specifically identified eosinophils by using an eosinophil-GFP reporter mouse that was generated by crossing the previously described eoCRE mice with a flox-stop-flox-GFP reporter strain. These animals were then used to examine eosinophil recruitment using high-speed, fluorescent intravital microscopy.

Results: PF-573228 prevented the expression of VCAM-1 and CCL26 expression in IL-4-stimulated human endothelial cells. As a result, eosinophil adhesion and transmigration were blocked. PF-572338 also prevented IL-4-induced VCAM-1 expression *in vivo*. Using eosinophil-GFP reporter animals, we showed that PF-573228 significantly decreased rolling flux, adhesion and emigration. Histological examination of the tissue supported these findings. PF-573228 also led to decreased clustering and shape change in

emigrated eosinophils.

Conclusions: A FAK inhibitor blocks eosinophil recruitment *in vitro* and *in vivo* by preventing the expression of the endothelial cell adhesion molecule VCAM-1.

Grant Support: This work was funded by a grant from the Canadian Institutes for Health Research (CIHR) and an infrastructure award from the Canadian Foundation for Innovation (CFI).

#29 DEXPRAMIPEXOLE EFFECTIVELY LOWERS BLOOD AND TISSUE EOSINOPHILS IN SUBJECTS WITH CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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Rationale: Dexpramipexole is an oral investigational drug serendipitously noted to lower blood eosinophils in prior clinical studies in amyotrophic lateral sclerosis.

Methods: An open-label study of dexpramipexole 300 mg/day was undertaken in subjects with chronic rhinosinusitis with nasal polyps (CRSwNP) with a baseline blood absolute eosinophil count (AEC) ≥0.30x10^9/L and polyp eosinophilia. The primary endpoint examined was change in AEC from baseline to end of study. Change in nasal eosinophils from baseline to end of study was an exploratory endpoint.

Results: Baseline AEC was 0.525x10^9/L in the 16 subjects who completed the study. AEC at month 6 was 0.03x10^9/L, a 94% reduction ($p<0.001$). Ten of the 16 subjects had eosinophil counts reduced to 0.020x10^9/L or less at month 6. In the 13 subjects who had biopsies, polyp tissue eosinophilia was reduced from 123 to 5.4 eosinophils per high-powered field, a 96% reduction from baseline ($p=0.0005$). Dexpramipexole was well tolerated with no drug-related serious adverse events. Five subjects elected to continue on a long-term extension study.

Conclusions: In sum, dexpramipexole is a well-tolerated orally-available drug with robust blood and tissue eosinophil-lowering activity. Given that eosinophil lowering by dexpramipexole is greater than or equal to that of current biologics, its clinical activity in asthma and hypereosinophilic syndromes is of great interest. Based on its oral administration, safety profile, and convenience, dexpramipexole has the potential for broad use among patient populations with eosinophil-associated disorders.

Study Support: Knopp Biosciences, LLC

#30 IL-3 DIFFERENTIALLY ACTIVATES EOSINOPHILS COMPARED TO IL-5 AND GM-CSF

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Background: The receptor for IL-5 shares the common ß signaling subunit with the IL-3 and GM-CSF receptors. Therefore, these three receptors possess many redundant functions and yet can signal via separate pathways and differentially regulate their specific α-chain subunits. We aimed to determine how IL-3 differentially stimulates eosinophils compared to IL-5 and GM-CSF.

Methods: Human peripheral blood and airway eosinophils were obtained from allergic subjects. Peripheral blood eosinophils were activated *in vitro* with cytokines. Airway eosinophils were obtained from bronchoalveolar lavages 48 h after an *in vivo* allergen challenge.

Results: IL-3 is more potent than IL-5 or GM-CSF in maintaining the ERK/p90S6K/RPS6 ribosome-directed signaling pathway, leading to increased protein translation of semaphorin-7A and the low affinity receptors for immunoglobulin-G (FcGR2B/C). IL-3-induced prolonged CD32 (FcGR2A/B/C) and αMß2 surface expression and activation, which drove eosinophils to strongly degranulate on immobilized and aggregated IgG. Importantly, all the differential changes in blood eosinophils induced by in vitro exposure to IL-3 occurred *in vivo* in airway eosinophils after an *in vivo* allergen challenge.

Conclusions: Compared to IL-5 and GM-CSF, IL-3 differentially activates eosinophils by prolonging intracellular signaling. Conse-

quently, IL-3 is a potent inducer of CD32- and αMß2-mediated eosinophil degranulation, and thus, has an important impact on eosinophil biology independently of IL-5 or GM-CSF.

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#31 RNA-SEQUENCING ANALYSIS OF LUNG PRIMARY FIBROBLAST RESPONSE TO EOSINOPHIL-DEGRANULATION PRODUCTS PREDICTS DOWNSTREAM EFFECTS ON INFLAMMATION, TISSUE REMODELING AND LIPID METABOLISM

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Background: Exaggerated fibroblast activation, accumulation and change of phenotype may lead to fibrosis and loss of tissue function. So far, little information has been reported on how eosinophils affect inflammation and tissue remodeling through the activation of fibroblasts, particularly lung fibroblasts. We propose to analyze the global function of IL-3-activated eosinophil degranulation products on human primary lung fibroblasts (HLF) using the whole transcriptome sequencing technology (RNA-seq).

Methods: Conditioned media from eosinophils pre-activated with IL-3 or IL-5, and degranulated for 6 h on heat-aggregated IgG were added on HLF for 24 h. HLF whole transcriptome sequencing was analyzed using the Ingenuity pathway Analysis (IPA), and was validated using qPCR.

Results: In fibroblasts, the expression level of 300 genes was changed by conditioned media from IL-3-activated eosinophils compared to control fibroblast cultures. Among these 300 genes, the expression level of 35 genes coding for known proteins was upregulated by IL-3- versus IL-5-activated eosinophils. Of the 35 upregulated genes, IPA identified C3, CH25H (cholesterol 25-hydroxylase), CXCL1 (GROα), CXCL8 (IL-8), CYP1A1 (Cytochrome P1-450), ICAM1, IL6 and UCN2 (ligand for corticotropin-releasing hormone receptor) as having downstream functions on the immune response, tissue remodeling and lipid synthesis. In addition, this analysis combined with previous RNA sequencing analyses of eosinophils suggest that IL-1ß, OSM (oncostatin M) and TNFSF12 (TWEAK) are the potential upstream regulators of fibroblasts.

Conclusion: This study has identified several novel pro-inflammatory and pro–remodeling mediators produced by fibroblasts in response to products released from activated eosinophils. The upstream (from eosinophils) and downstream (from fibroblasts) mediators identified in our study should be further validated and examined to better understand the tissue pathologies related to the eosinophil/fibroblast interaction.

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#32 EOSINOPHILS IMPAIR AIRWAY SUBSTANCE P BREAKDOWN BY INHIBITING NEUTRAL ENDOPEPTIDASE

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Background: Asthma is a heterogeneous disease with airway inflammation that may be eosinophilic or neutrophilic. Different inflammatory phenotypes may contribute to airway nerve dysfunction by changing the expression or degradation of neurotransmitters. Substance P is a neuropeptide that evokes cough, bronchoconstriction, inflammation, and vascular leakage. Thus we sought to define the effects of eosinophils on nerve substance P expression and its contribution to airway reactivity.

Methods: Eosinophil-deficient ((-)Eos) and wild-type (WT) mice were sensitized and challenged intranasally with house dust mite antigen (HDM). Animals were mechanically ventilated and the increase in airway resistance in response to inhaled serotonin was measured. Evan's Blue dye was injected intravenously prior to serotonin dose-response curves to assess vascular leakage. In some animals, substance P breakdown by neutral endopeptidase was inhibited with phosphoramidon. Bronchoalveolar lavage substance P was measured with ELISA. Whole mount tracheas were immunofluorescently labeled using antibodies to the pan-neuronal marker PGP9.5 and to substance P. Tracheas were optically cleared and confocal images were used to construct three-dimensional computer models of airway nerves to calculate substance P positive nerve volume.

Results: HDM exposure increased eosinophils in airway lavage fluid in WT mice ($p<0.0001$) compared with saline controls. In contrast, in (-)Eos mice, HDM exposure increased lavage neutrophils ($p=0.02$). HDM exposure increased serotonin-induced bronchoconstriction in WT mice ($p<0.0001$), but not in (-)Eos mice. Vagotomy decreased and atropine blocked serotonininduced broncho constriction. In both WT and (-)Eos mice, HDM increased both neuronal ($p=0.0004$) and airway lavage ($p<0.0001$) substance P.

Blocking neutral endopeptidase activity increased bronchoconstriction in (-)Eos HDM-treated mice, but not in WT HDM-treated mice. Correspondingly, lavage neutral endopeptidase activity was suppressed by HDM treatment in WT mice, but not in (-)Eos mice. Vascular leakage was greater in (-)Eos HDM-treated mice compared with WT HDM-treated mice.

Conclusion: Airway inflammation increases substance P levels, but only eosinophilic inflammation decreases neutral endopeptidase, impairing degradation of substance P. This may contribute to increased reflex bronchoconstriction in asthma. Neutrophilic inflammation was associated with increased vascular leakage and may contribute to airway edema in asthma.

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#33 QUANTIFICATION OF 'WHOLE LUNG' EOSINOPHILIC INFLAMMATION IN ASTHMA AND SYSTEMIC DISEASE USING RADIOLABELLED AUTOLOGOUS HUMAN EOSINOPHILS

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Background: Eosinophils are key mediators of allergic inflammation. The ability to localise and quantify eosinophilic inflammation in vivo is an important clinical goal, and would support the evaluation of eosinophil-targeted therapeutics. We aimed to quantify eosinophil distribution and organ-specific uptake in healthy subjects, patients with asthma and those with focal pulmonary eosinophilic inflammation.

Methods: We injected autologous, peripheral blood-derived technetium-99m- or indium-111-labelled eosinophils intravenously into 8 healthy volunteers, 15 asthmatics, and 3 patients with focal eosinophilic inflammation, and monitored eosinophil distribution using dynamic planar imaging, single photon emission computed tomography (SPECT)/CT, and whole body counting. Lung accumulation of technetium-99m-labelled eosinophils was quantified using Patlak-Rutland analysis. Whole body indium-111-labelled eosinophil distribution and loss were assessed using a whole body counter in a separate cohort of 5 healthy volunteers and 7 asthmatics.

Results: Pulmonary eosinophil clearance (slope/intercept of Patlak-Rutland plot) was increased in patients with focal eosinophilic inflammation (0·0033 ml/min/ml; 95% CI -0·005–0·011; p=0·02) and asthma (0·0007 ml/min/ml; 95% CI 0·0003–0·0010; p=0·14) compared to healthy volunteers (0·0003 ml/min/ml; 95% CI -7·5x10-5–0·0008). Absolute eosinophil migration (eosinophil clearance multiplied by blood eosinophil count) was also significantly elevated in patients with focal inflammation (5932 eosinophils/min/ml; 95% CI -14351–26215; p=0·01) and asthma (364 eosinophils/min/ml; 95% CI 38–689; p=0·03) versus healthy volunteers (38 eosinophils/min/ml; 95% CI -11–87). However, we detected no differences in absolute eosinophil loss from the body between asthmatics and healthy volunteers over the 10-day study period.

Conclusions: The use of radiolabelled eosinophils coupled with SPECT/CT can quantify pulmonary eosinophilic inflammation in humans, with the potential for patient endotyping and testing novel eosinophil-targeted treatments.

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#35 INCREASED BEIGE FAT AND EOSINOPHIL INFILTRATION IN MICE LACKING KRÜPPEL-LIKE FACTOR 3 (KLF3)

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Background: This study aims to explore the role of Krüppel-like factor 3 (KLF3), a transcriptional repressor, in the recruitment of beige fat

Methods: The blood and adipose tissues of KLF3 knockout mice have been characterised extensively. Circulating and adipose-resident blood cell counts have been undertaken, including a more in-depth analysis of eosinophils and macrophages within the subcutaneous adipose tissue. We have assessed immune cell infiltration, mitochondrial content, gene and protein expression, and have commenced studies involving response to environmental stimuli in the form of cold exposure and activation of the β3-adrenoreceptor.

Results: Mice lacking the transcriptional repressor, KLF3, are lean, resistant to obesity, and have smaller fat pads. Interestingly, we observed that the diminished subcutaneous fat of KLF3 knockout mice also has a browner appearance, reminiscent of enhanced

beige fat biogenesis in a process known as adipose "browning". Closer examination of the subcutaneous fat showed increased expression of important beige fat markers - most notably the electron transport chain uncoupler, Uncoupling protein 1 (*Ucp1*), which is required for thermogenesis. We assessed the stromal vascular fraction of subcutaneous adipose, and found greatly increased infiltration of eosinophils, which are known to be involved in the browning process via their recruitment and maintenance of beige fat. Isolation of these resident eosinophils by FACS allowed us to perform microarrays to investigate changes in cellular gene expression. We identified several key genes, including interleukin-33 (*Il33*) and meteorin-like (*Metrnl*), which are significantly up-regulated in fat-derived eosinophils lacking KLF3. These results have provided a starting point for further investigation of direct KLF3 targets in eosinophils.

Conclusions: Mice lacking KLF3 are lean, have reduced adiposity and enhanced recruitment of beige fat in their subcutaneous adipose depot. This may be due to an increased infiltration of blood cells involved in maintaining energy homeostasis in fat, namely eosinophils.

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#36 LOCAL IL-5 PRODUCTION BY BONE MARROW ILC2s IN IL-33-DRIVEN EOSINOPHILIA

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Background: Type 2 innate lymphoid cells (ILC2s) have been found to produce large amounts of type 2 cytokines, including interleukin (IL)-5, in response to IL-33. Several studies have demonstrated the importance of IL-33 in eosinophilic airway inflammation. Although ILC2s have emerged as important producers of IL-5 in airways, the cellular source of IL-5 in the bone marrow is less explored. In this study, we tested the hypothesis that IL-33-induced airway eosinophilia is an IL-5-dependent process in which ILC2s produce IL-5 locally in the bone marrow.

Method: IL-5 production by bone marrow ILC2s was analyzed by intracellular flow cytometry in a murine model of IL-33-induced airway eosinophilia.

Result: Intranasal IL-33 administration resulted in eosinophil infiltration in airways and increased eosinophils in bone marrow. This was accompanied by a dramatic induction of eotaxin-2/CCL24 in airways, indicating eosinophil recruitment to the tissue. Systemic levels of IL-5 increased upon IL-33 treatment, and airway and bone marrow eosinophils were significantly reduced in mice pretreated with anti-IL-5 antibodies followed by intranasal IL-33. Interestingly, IL-5+ ILC2s as well as expression of the IL-33 receptor (ST2) on ILC2s were greatly increased in the bone marrow in response to IL-33.

Conclusion: Our findings demonstrate that IL-5 is crucial for IL-33-mediated eosinophilia in vivo, a process in which ILC2s constitute a local source of IL-5 in the bone marrow.

Keywords: IL-33, ILC2, IL-5, Bone marrow, Eosinophilia

#37 EOSINOPHILS SECRETE GALECTIN-10 *VIA* EOSINOPHILIC EXTRACELLULAR TRAPS, EXOSOMES AND IMMUNE SYNAPSES

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Galectin-10 has been shown to mediate the T cell suppressive function of regulatory T cells. Eosinophils have T cell suppressive capacity and contain large amounts of galectin-10, but it is largely unknown how galectin-10 is released from eosinophils. Our primary goal was to determine how galectin-10 is released from eosinophils exposed to proliferating T cells. Human eosinophils were cocultured with CD3/CD28-activated T cells for 2 days and the cells were then stained for CCR3, DAPI, galectin-10, CD9 and CD63 and analyzed with Image flow cytometry. We discovered that eosinophils cocultured with proliferating T cells released DNA-containing eosinophil extracellular traps with punctate deposits of galectin-10. When DNase was added to the cocultures the T cell suppressive function of eosinophils was reverted. In order to determine if the DNA in the extracellular traps was of nuclear or mitochondrial origin, staining with a fluorescent mitochondrial stain and mAb against histones revealed that the DNA was derived from the nucleus. Additionally, immune synapses containing galectin-10 were seen between T cells and eosinophils as well as $CD9^{+}CD63^{+}$ exosomes containing galectin-10. To conclude, eosinophils appear to release galectin-10 via at least three different mechanisms when exposed to proliferating T cells.

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#38 CLINICAL SIGNIFICANCE AND ANTIGENIC SPECIFICITY OF ANTI-EOSINOPHIL AUTOANTIBODIES

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While there is abundant literature on anti-neutrophil cytoplasmic auto-antibodies (ANCA), there are so far only two articles published on the existence of anti-eosinophilic auto-antibodies (AEOSA). AEOSA have been closely associated with primary biliary cholangitis and more loosely associated with other auto-immune conditions affecting the liver, kidneys and joints. The detection of AEOSA is however not yet used to predict or diagnose any disease. In the region of Gothenburg (Sweden, 500'000 inhabitants), 20-30 patients per year display positive auto-immune reaction to eosinophils. The goal of the current study is to determine if the detection and the antigenic specificity of AEOSA are of clinical use.

In the Department for Clinical Immunology, around 1% (27/3000) of the sera tested for the presence of ANCA by immunofluorescence were positive for AEOSA. The AEOSA were seen in combination with ANCA (11/27, 40%) or in absence of ANCA (16/27, 60%). The main auto-antigen recognized by AEOSA was the protein Eosinophil Peroxidase (EPX) (18/27, 66%). AEOSA+/ANCA+ sera were reacting slightly more often against EPX (8/11, 72%) than AEOSA+/ANCA- sera (10/16, 62.5%). Remarkably, autoantibodies against EPX were still detectable in 9/14 (64%) patients at the 1-year follow-up. Other eosinophilic antigens like Eosinophil Cationic Protein (ECP) were also identified as target of AEOSA. The clinical symptoms associated with the presence of AEOSA in our cohort varied. Liver and kidney inflammatory disease as well as auto-immune diseases affecting the thyroid, gastrointestinal tract and joints are overrepresented in our cohort, with over 25% occurrence of one or more of these pathologies diagnosed in patients positive for AEOSA in immunofluorescence.

We confirm that eosinophil peroxidase, like myeloperoxidase and thyroid peroxidase, is a target of auto-antibodies, illustrating the high antigenic potential of peroxidases. The significance and the pathogenic potential of AEOSA is still under investigation.

#39 INVESTIGATION OF NATURAL KILLER CELL–MEDIATED EOSINOPHIL APOPTOSIS BY BENRALIZUMAB

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Background: Increased eosinophils in the airways of patients with asthma correlate with increased disease severity, airway obstruction, and susceptibility to exacerbations. Benraluzumab is a humanized, afucosylated monoclonal antibody that targets the IL-5 receptor alpha (IL-5Rα) antigen predominantly expressed on eosinophils and basophils. Lack of fucose (afucosylation) increases the affinity to human Fcγ-receptor IIIa (CD16) and significantly enhances antibody-dependent cell-mediated cytotoxicity (ADCC). In patients with asthma, benralizumab potently and rapidly depletes eosinophils in peripheral blood, bone marrow and within the airway mucosa and sputum. Benralizumab has been well-tolerated and demonstrated clinical efficacy in in two pivotal Phase III trials[1,2], resulting in reductions in annual asthma exacerbation rates compared with placebo for patients with severe, uncontrolled asthma across a range of baseline blood eosinophil counts. In addition, benralizumab improved lung function, (FEV_1), and health-related quality of life, (i.e., ACQ-6 scores), compared with placebo. We have previously demonstrated that in the presence of natural killer (NK) cells, benralizumab-induced significant eosinophil and basophil apoptosis *in vitro*, as assessed by Annexin V+ staining. We also found that eosinophil apoptosis induced by benralizumab was not associated with significant eosinophil degranulation. Here we further characterize the mechanisms underlying NK-mediated killing of eosinophils targeted with benralizumab. In these studies, we have employed live cell fluorescence imaging to determine mode of action *in vitro* using primary human eosinophils and NK cells.

Methods: NK cells were loaded with lysotraker yellow to label acidified organelles and stained with green fluorescent dye while eosinophils (target cells) were labeled with the far-red fluorescent dye. Eosinophils and NK cells were then co-incubated at a ratio of 1:3 in the presence of benralizumab or the control isotype. A blue nucleic acid stain POPRO-1 dye was used to detect apoptosis. ADCC was imaged on a Leica SP5 laser scanner confocal microscope equipped with x63 oil objective heater and temperature was maintained at 37°C.

Results: We illustrated the steps of NK-mediated killing of eosinophils targeted with benralizumab. NK-eosinophil interactions were imaged every 60s for 120 min via life cell confocal microscopy. Imaging was performed in the presence of POPRO-1 dye, which

does not permeate the membrane of living cells and thus identifies dying cells within the population. Following initial contact with eosinophils, the killing process was initiated by the migration of NK lytic granules to the cellular surface, a step defined as granule polarization. Subsequently, these granules fused with the eosinophil membrane resulting in delivery of cytolytic mediators at the cellular interface inducing eosinophil membrane blebbing and nuclear collapse. Although the granule polarization occurred rapidly, the initiation of eosinophil death, as marked by POPRO-1 entry, was not visualized until approximately 2 h later and increased over time.

Conclusions: Collectively, our data confirm that NK cells can mediate benralizumab-induced killing of eosinophils and will thus provide deeper insights into the apoptotic process via which these cells die.

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#40 HIGH AFFINITY IGE RECEPTOR ENHANCES *IN VIVO* ANTIGEN PRESENTATION CAPABILITIES OF AIRWAY EOSINOPHILS

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Background: The functional significance of high affinity IgE receptors (FcεRI) expressed by eosinophils from patients with allergic diseases is still unclear. We evaluated the role of FcεRI in eosinophil antigen presentation *in vivo* by using humanized FcεRI α chain (hFcεRIα) transgenic mice.

Methods: Peritoneal eosinophils from humanized FcεRI mice were allowed to process antigen NP-OVA *in vitro*, with or without chimeric anti-NP IgE targeting to eosinophil hFcεRI, and then were instilled intratracheally into NP-OVA immunized recipient mice.

Results: By flow cytometric analysis, the instilled hFcεRI-bearing eosinophils trafficked from the airway into the draining paratracheal lymph nodes, where these donor eosinophils pretreated *in vitro* with NP-OVA and anti-NP IgE complexes presented antigen to T cells more effectively than those pretreated with NP-OVA only, as assayed by paratracheal lymph node T cell proliferation. IgE/FcεRI-facilitated eosinophil antigen-presenting function resulted in increasing IL-4 but not INF-γ production by paratracheal lymph node T cells. In addition, cross-linking of hFcεRI on airway eosinophils increased their expression of co-stimulatory molecules CD40, CD80 and CD86.

Conclusions: These data suggest that the hFcεRI on murine eosinophils can enhance their antigen-presenting function by not only facilitating antigen capturing, processing and presenting, but also increasing expression of co-stimulatory molecules.

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#41 POTENT EOS ADHESION WITH IL-33 STIMULATION AND PERIOSTIN AS SUBSTRATE

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Background: IL-33 and periostin have been recently described as important biomarkers and mediators in asthma. IL-33, a member of the IL-1 family, is produced by airway epithelial cells, fibroblasts, and endothelial cells. Increased IL-33 is associated with Th2 inflammation in allergic disease and asthma and it has recently been shown to activate EOS and prolong their survival. Periostin is a secreted matricellular protein involved in cell proliferation, cell invasion and tissue remodeling. It also plays a role in asthma, including EOS recruitment, airway remodeling, and development of a Th2 phenotype. Traditional assessment of EOS adhesion used IL-5 stimulation and adhesion to integrins such as VCAM-1 or ICAM-1. We sought to determine the relative potency of IL-33 and periostin in EOS adhesion.

Methods: Fresh blood EOS were prepared in our EOS Laboratory Core from human subjects 18-55 yo, most with a diagnosis of allergic rhinitis with or without mild asthma. EOS were plated onto 96 well plates coated with ICAM-1, VCAM-1, or periostin and were stimulated with IL5, IL3, GMCSF, or IL33. Eosinophil peroxidase activity was measured from cells that remained adhered to the plate after incubation at 37C for 30min and washing with Hanks balanced salt solution. Adhesion was calculated as a percentage of total wells containing lysed EOS.

Results: Adhesion was compared upon stimulation of EOS with IL-5 and IL-33. With ICAM-1 as substrate, IL-33 stimulated adhesion (26% +/- 13%) was significantly greater than IL-5 stimulation (12% +/- 7%, p=0.003). Similarly, with periostin as substrate, IL-33

stimulated adhesion (45% +/- 11%) was significantly greater than IL-5 stimulation (29% +/- 12%, p=0.002). Adhesion of EOS was also compared on different substrates. IL-5 stimulated adhesion on VCAM-1 (33% +/- 14%) was greatest, followed by periostin (20% +/- 8%) and ICAM-1 (3% +/- 3%). IL-33 stimulated adhesion was also greatest on VCAM-1 (29% +/- 12%), followed by periostin (25% +/- 12%) and ICAM-1 (6% +/- 5%).

Conclusion: These data indicate that some of the more recent biomarkers associated with asthma are potent activators of EOS. It is thought that both IL-33 and periostin exposure will occur within the lung tissue and may serve as co-stimulation for EOS activation.

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#42 EOSINOPHIL ACCUMULATION IN OXAZOLONE-INDUCED ATOPIC DERMATITIS IS INDEPENDET OF IL-13 RECEPTOR ALPHA 1

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Background: IL-13 and IL-4 are potent mediators of type 2-associated inflammation such as those found in asthma and atopic dermatitis. We have previously shown that the cardinal features of allergic asthma including airway hyperresponsiveness, mucus production and fibrosis are exclusively dependent on the type 2 IL-4 receptor (R), which is comprised of IL-4Rα and IL-13Rα1. Nonetheless, the role of IL-13Rα1 in atopic dermatitis is unknown.

Objective: To define the role of IL-13Rα1 in experimental atopic dermatitis

Methods: Wild type and *Il13ra1*$^{-/-}$ mice were sensitized on day 0 by the topical administration of 15µl of 1% oxazolone in acetone on both aspects of each ear. Starting on day 7, mice were challenged with 0.5% oxazolone three times a week to a total of 9 challenges. Thereafter, ear thickness and histopathology were assessed by a digital caliper and histology, respectively. In addition, ears were enzymatically digested and infiltration of inflammatory cells was determined by polychromatic flow cytometry. Expression of Th2-associated cytokines and chemokines in the skin was determined by qPCR and serum IgE levels were measured by ELISA.

Results: Oxazolone-treated wild type mice displayed markedly increased ear thickness and severe histopathology. In sharp contrast, *Il13ra1*$^{-/-}$ mice were nearly completely protected from oxazolone-induced ear thickening and displayed markedly decreased pathology. Assessment of infiltrating immune cells into the skin revealed markedly elevated levels of multiple cells including neutrophils, eosinophils, monocytes, macrophages, and lymphocytes in oxazolone-treated wild type mice. Interestingly, while cellular influx into oxazolone-treated *Il13ra1*$^{-/-}$ was significantly decreased compared to oxazolone-treated wild-type mice, eosinophil numbers in the skin were comparable. Assessment of type 2 cytokine-associated cytokines such as IL-4 revealed no difference in type 2 cytokine levels between oxazolone-treated wild type and *Il13ra1*$^{-/-}$ mice. Surprisingly, despite similar levels of IL-4, oxazolone-treated *Il13ra1*$^{-/-}$ mice exhibited higher serum IgE titers.

Conclusions: Our data demonstrate that IL-13Rα1 mediates the major clinical features of oxazolone-driven dermatitis and reveal an IL-13Rα1-independent mechanism for recruitment of eosinophils into the skin. These data have significant implications and highlight IL-13Rα1 as a potential therapeutic target in dermatitis.

#43 EOSINOPHIL PERSISTENCE IN THE AIRWAY FOLLOWING *ALTERNARIA ALTERNATA* INHALATION EXPOSURE

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Background: Eosinophils in the airways and bronchial biopsies are a hallmark of the steroid resistant / eosinophilic asthma phenotype, and controlling eosinophils in this condition has been a successful therapeutic measure [Haldar *et al*., 2009; Nair *et al*., 2009]. Here we feature a mouse model of allergic airways disease that utilizes intranasal challenge with a fungal extract, as this approach may more closely resemble the type of environmental stimulus that invokes as asthmatic response in human subjects.

Methods: Mice include *Rag1*$^{-/-}$, *IL-13*$^{-/-}$, *IL-4*$^{-/-}$ and *GM-CSF*$^{-/-}$ in addition to wild-type strains. Mice were subjected to intranasal challenge only (no sensitization) with extracts from *A. alternata* (*Aa*; 50 µg in 50 µL pbs) or pbs diluent control at days 0, 3 and 6 followed by evaluation at various time points thereafter. Leukocyte differentials were determined via visual inspection of cytospins from bronchoalveolar lavage (BAL) fluid stained with modified Giemsa. Eosinophils ($CD45^+CD11c^-SiglecF^+$) were identified in single cell suspensions from whole lung by flow cytometry. Cytokines in BAL fluid were identified via Proteome profiler screen and/or ELISA (R&D Systems).

Results: No eosinophils were detected in the airways at 24 hrs after the first *Aa* challenge, and few eosinophils were detected after the second challenge. Prominent eosinophil recruitment was observed after three inoculations (20 - 40% of total leukocytes), and eosinophils persisted in the airways at this level with no further exogenous stimulation through day 24. Eosinophil recruitment in response to *Aa* inhalation challenge is fully dependent on T lymphocytes; little to no recruitment is observed in *Rag1*$^{-/-}$ mice. Furthermore, individual deficiencies of IL-4 or IL-13 impaired eosinophil recruitment in response to *Aa* inhalation and no sustained viability was observed. By contrast, absence of the pro-survival cytokine, GM-CSF, had no impact on eosinophil recruitment or on sustained viability in response to *Aa* challenge. Eosinophils isolated from the airways of *Aa*-challenged mice display normal morphology and staining properties, although those isolated from lung tissue of *Aa*-challenged mice display a 2 - 3 fold increase in MFI (median fluorescence index) for the characteristic cell surface antigen Siglec F compared to those isolated from control-challenged mice; this value reaches a peak at day 7 immediately after the final *Aa* inoculation. A full screen of cytokines in the BAL fluid of *Aa*-challenged mice revealed elevated levels of IL-5 and IL-13 through day 10 and 17 respectively, while IL-3 and IL-27 remained undetectable throughout. As such, there are no cytokine expression patterns that can fully account for the prolonged viability of eosinophils in the airways and in the lung tissue. Of interest, recent findings from our laboratory (Geslewitz *et al*., unpublished) suggest that allergen challenge may alter eosinophil physiology and permit survival in the absence of exogenous cytokine support.

Conclusions: Future therapies designed to limit the negative impact of eosinophils may need to consider not only recruitment to the lungs but also independent mechanisms underlying sustained viability.

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#44 EOSINOPHILS AND ALTERED SKIN BARRIER FUNCTION IN DEVELOPMENT OF FOOD ALLERGY: NOVEL MECHANISMS OF FOOD ALLERGY

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Background: The mechanisms of food allergy development are unknown but are clearly linked in patient populations to a genetic predisposition towards skin barrier defects. Whether this functionally contributes to development of food allergy is unknown. We determined novel mechanisms for development of food allergy without the use of adjuvant.

Methods: At postnatal days 3 to 19, offspring, that were heterozygous for defects in skin barrier genes and that were from allergic or non-allergic wild type mothers, were sensitized by 3 to 7 cutaneous exposures to environmental allergens and the food allergens peanut or chicken egg ovalbumin (OVA). Then, offspring received oral gavage with allergen and anaphylaxis was determined by monitoring for decreased body temperature. Tissues were collected for analysis of inflammation and inflammatory mediators.

Results: Food allergen-induced anaphylaxis was induced in neonatal mice following concurrent cutaneous neonatal sensitization to food and environmental allergens. The sensitization induced increased serum IgE and a robust eosinophilia in the skin but did not change skin mast cell numbers. Moreover, maternal sensitization to OVA transmitted elevated offspring responsiveness to peanut sensitization and challenge. For offspring of allergic mothers, ≤3 skin sensitizations were required and, for offspring of non-allergic mothers, 4 skin sensitizations were required for oral food-induced anaphylaxis. Importantly, responsiveness in neonatal mice was dependent on genetic defects in skin barrier function that developed in the absence of any adjuvant.

Conclusions: These studies document mechanisms for development of food allergy in mice that are consistent with features of early life exposures and genetics in clinical food allergy and demonstrate that changes in barrier function are required for the development of anaphylaxis to food allergen.

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#45 SHORT CHAIN FATTY (SCFA) ACIDS ACTIVATE THE INTRINSIC APOPTOSIS PATHWAY IN EOSINOPHILS

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Background: The increasing incidence of allergic inflammatory diseases points out the growing necessity for eosinophil-targeting therapeutics. Short chain fatty acids, e.g. acetate, propionate and butyrate are produced in high concentration in the gastro-intestinal tract by commensal bacteria and are readily secreted into the blood stream and thereby show various biological functions. Prompted by the observation that propionate hampers lung eosinophilia in models of allergic inflammatory diseases we hypothesize that SCFA modulate the survival of eosinophils.

Methods: Induction of apoptosis was detected using annexin V/propidium iodide (PI) double staining, JC-1 staining and caspase 3/7 activation assay. mRNA expression was detected via real-time RT-PCR.

Results: We found that both, propionate and butyrate induce apoptosis in human peripheral blood eosinophils, starting 18 h after the initial treatment ($p<0.001$ and $p<0.01$, 1-way ANOVA, Bonferroni post-test). This result was confirmed via the reduction of the

mitochondrial membrane potential, as detected with JC-1 staining ($p<0.05$ and $p<0.01$, 1-way ANOVA, Bonferroni post-test) and Caspase 3/7 activation assay ($p<0.001$ and $p<0.05$, 1-way ANOVA, Bonferroni post-test). These findings suggest an involvement of the intrinsic apoptotic pathway in eosinophils. Additionally, IL-5 pretreatment could not prevent the activation of caspase 3/7 as induced by propionate or butyrate (both $p<0.001$, 1-way ANOVA, Tukey post-test. Furthermore, IL-5RA transcript was downregulated after incubation with propionate or butyrate for 3 h (both $p<0.001$, Bonferroni post-test).

Conclusions: We could show for the first time that the SCFA propionate and butyrate are able to interfere with survival pathways in eosinophils, in terms of induction of apoptosis, mitochondrial depolarization and activation of effector caspases. Crucially, this effect could not be prevented by IL-5 pretreatement. Therefore, we propose that propionate and butyrate could serve as potential therapeutic agents in allergic inflammatory diseases.

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#46 I WILL SURVIVE: LUNG EOSINOPHILS ISOLATED FROM ALLERGEN-CHALLENGED MICE DISPLAY PROLONGED VIABILITY IN THE ABSENCE OF IL-5

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Background: Eosinophil recruitment to the lung is a hallmark of eosinophil-associated asthma; therefore, it would be helpful to understand the mechanisms by which eosinophils are not only recruited, but sustained, in this environment. Sensitization and challenge with extracts of the fungus, *Aspergillus fumigatus* (*Af*), is commonly used as a murine model of allergic airways disease as eosinophils are recruited to the respiratory tract in response to cytokine provocation. In this study, we explore mechanisms that maintain eosinophil viability both *in situ* as well as *ex vivo*.

Methods: Wild-type BALB/c mice were sensitized with two intraperitoneal injections of *Af* and alum adjuvant, then subsequently challenged with three intranasal *Af* inoculations. Eosinophils were isolated from single cells suspensions by negative selection via magnetic-activated cell sorting (purity > 90%). The lung-derived eosinophils were placed in growth media (RPMI 1640 + 20% fetal calf serum, with or without 5 ng/mL IL-5), and maintained at 37°C + 5% CO_2. The viability of these eosinophils was compared to those isolated from otherwise untreated, IL-5 transgenic mice cultured under identical conditions. Viability was measure by visual counting sample from each group every 24 hours for 5 days with trypan blue staining.

Results: As anticipated, when supplemented with IL-5, all isolated eosinophils maintain viability *ex vivo*; at t = 72 hours, eosinophils from *Af* sensitized and challenged mice and eosinophils isolated from IL-5 tg mice are 74% and 67% viable, respectively. However, and surprisingly, eosinophils isolated from lungs of *Af* sensitized and challenged mice maintain significantly higher long-term viability in the absence of supplemental IL-5 than do the lung eosinophils isolated from BALB/c IL-5 transgenic mice. At t = 24, 48, 72, and 96 hours, these eosinophils maintain an average viability of 77%, 64%, 72%, and 57%, respectively. This is in contrast to eosinophils isolated from IL-5 transgenic mice, which maintain viability of lower than 10% at day 5 in the absence of cytokine support.

Conclusions: Our findings indicate that sensitization and challenge with *Af* not only promotes migration of eosinophils into the lung, but the eosinophils themselves undergo physiologic activation which results in prolonged survival in the absence of recognized anti-apoptotic cytokines. As such, we will need to explore the mechanisms underlying this activation and prolonged survival and explore this as a more precise mechanism of asthma treatment.

Funding: NIAID Division of Intramural Research #AI000941 to HFR

#47 ALTERED miR-155 EXPRESSION IN ALLERGIC ASTHMATIC AIRWAYS

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Background: We and others have previously identified microRNAs (miRNAs) with pathological roles in animal models of asthma. To date, few studies have investigated miRNA expression in human asthmatics. However, both miR-146a and miR-155 have been described to play important roles in the regulation of inflammatory responses.

Methods: Induced sputum and blood samples were obtained from allergic asthmatics and healthy controls. Peripheral blood mononuclear cells (PBMCs) were stimulated with αCD3/CD28. RNA was purified from (PBMCs), sputum supernatants or sorted sputum monocytes and lymphocytes collected in and out pollen season.

Results: Significantly lower levels of miR-155 were detected in cell-free sputum from allergic asthmatics compared to healthy controls. Induced sputum isolated from allergic asthmatics in and out of pollen season demonstrated increased eosinophil numbers in season. Interestingly, miR-155 expression, but not miR-146a, was reduced in sputum lymphocytes in season compared to post-season. Furthermore, miR-155 was found to increase whereas miR-146a decreased in PBMCs and cell-free PBMC culture media upon T cell receptor stimulation via αCD3/CD28 both in allergic asthmatics and healthy controls.

Conclusion: Our findings suggest that miR-155 is differentially expressed *ex vivo* in airways in allergic asthmatics compared to healthy controls, which may have implications in the local immune response in allergic asthma.

#48 EVALUATION OF IMMUNOHISTOPATHOLOGIC AND THERAPEUTIC EFFECTS OF ANTI SIGLEC-F ANTIBODY IN THE LUNG OF A MOUSE MODEL OF BREAST CANCER AND ALLERGIC ASTHMA

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Background: exploring the role of eosinophils in cancer and metastasis.

Methods: we generated a mouse model of allergic asthma with breast cancer and administered those mice anti-Siglec-F (Sialic Acid-Binding Immunoglobulin-Like Lectin F) antibody to cause apoptosis in eosinophils and explore the role of eosinophils in cancer and methastasis.

Allergic asthma was induced via injection and nebulization of Ovalbumin. Breast cancer was induced by subcutaneous injection of 4T1 cell line. Eight different groups of mice were used in this study.

Results: In cancer group, administration of anti-Siglec-F Ab caused significant increase of IL-4 and IL-5 level and significant reduction of IL-10 level compared with cancer group received PBS. In asthma group administration of anti-Siglec-F Ab significantly increased the level of IL-4, IL-5 and IL-12 and diminished the level of IL-6 and IL-10. In asthma and cancer group administration of Sieglec F Ab leads to increased level of IL-4, -5, -6, -10 and -12 cytokines. Interestingly administration of Ab significantly decreased OVA specific IgE level in sera of both asthmatic and asthma and cancer mice.

Pathological evaluation of lungs from animals received antibodies showed extensive metastasis in cancer group which was associated with 25% mortality in mice however antibody administration in asthma and cancer mice revealed significantly decreases in metastatic foci.

Conclusions: Our data showed that eosinophils behave differently in cancer and in asthma environment, therefore administration of anti siglec-F antibody had different outcome our experimental groups.

Grant Support: This study was funded by a grant from Tehran University of Medical Sciences (Grant No: 227.301.053)

#49 STAT6 AND SOCS3: INVOLVEMENT IN CCL26 PRODUCTION BY BRONCHIAL EPITHELIAL CELLS IN ASTHMA AND ITS SEVERITY

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Background: High pulmonary eosinophil counts correlate with asthma severity and exacerbation. We recently showed that sputum CCL26 levels correlate with sputum eosinophils. We also found that among all CC chemokines, IL-13 selectively induced the expression of CCL26 by bronchial epithelial cells (BECs) and this phenomenon is significantly enhanced in BECs from severe eosinophilic asthmatics. We postulated that the superior CCL26 production that we observed in severe asthmatics was the consequence of increased signaling events mediated by IL-13 and lower methylation levels in CCL26 promoter. We thus assessed the expression and functional responses of the different signaling effectors linked to the IL-13 signaling in CCL26 expression and methylation levels in CCL26 promoter in BECs from healthy subjects, mild asthmatics, and severe eosinophilic asthmatics.

Methods: Human primary BECs were isolated and cultured from bronchial biopsies. BECs were treated with IL-13 or vehicle for different times. Inhibitors or their vehicles were added to BECs 1 hour before IL-13. CCL26 expression was assessed by qPCR and ELISA. STAT6 and phosphorylated (p)STAT6 were quantitated by ELISA in BEC lysates. SOCS3 level were assessed by immunohistochemistry in bronchial biopsies. Methylation levels of five CpG in CCL26 promoter were quantitated by pyrosequencing in BECs.

Results: We confirmed the involvement of STAT6 in the induction of CCL26 expression by treating BECs with increasing STAT6 inhibitor. This led to a concentration-dependent inhibition of CCL26 expression in IL-13-stimulated BECs. In that regard, the pSTAT6/STAT6 ratios were increased in IL-13-stimulated BECs from severe eosinophilic asthmatics, compared to those from healthy subjects and mild asthmatics. This increased activation of STAT6 might be explained by a trend decrease of SOCS3 level observed in BECs from bronchial biopsies of severe eosinophilic asthmatics. Also, we demonstrated that methylation levels near to the STAT6 binding site in CCL26 promoter were similar in BECs of healthy controls and asthmatics.

Conclusions: Our results show the importance of STAT6 and the possible implication of SOCS3 in the enhanced CCL26 expression by BECs from severe eosinophilic asthmatics, and support their possible involvement in airway eosinophilia. (Supported by the Fondation de l'IUCPQ and the JD-Bégin Research Chair, and the CIHR doctoral scholarship).

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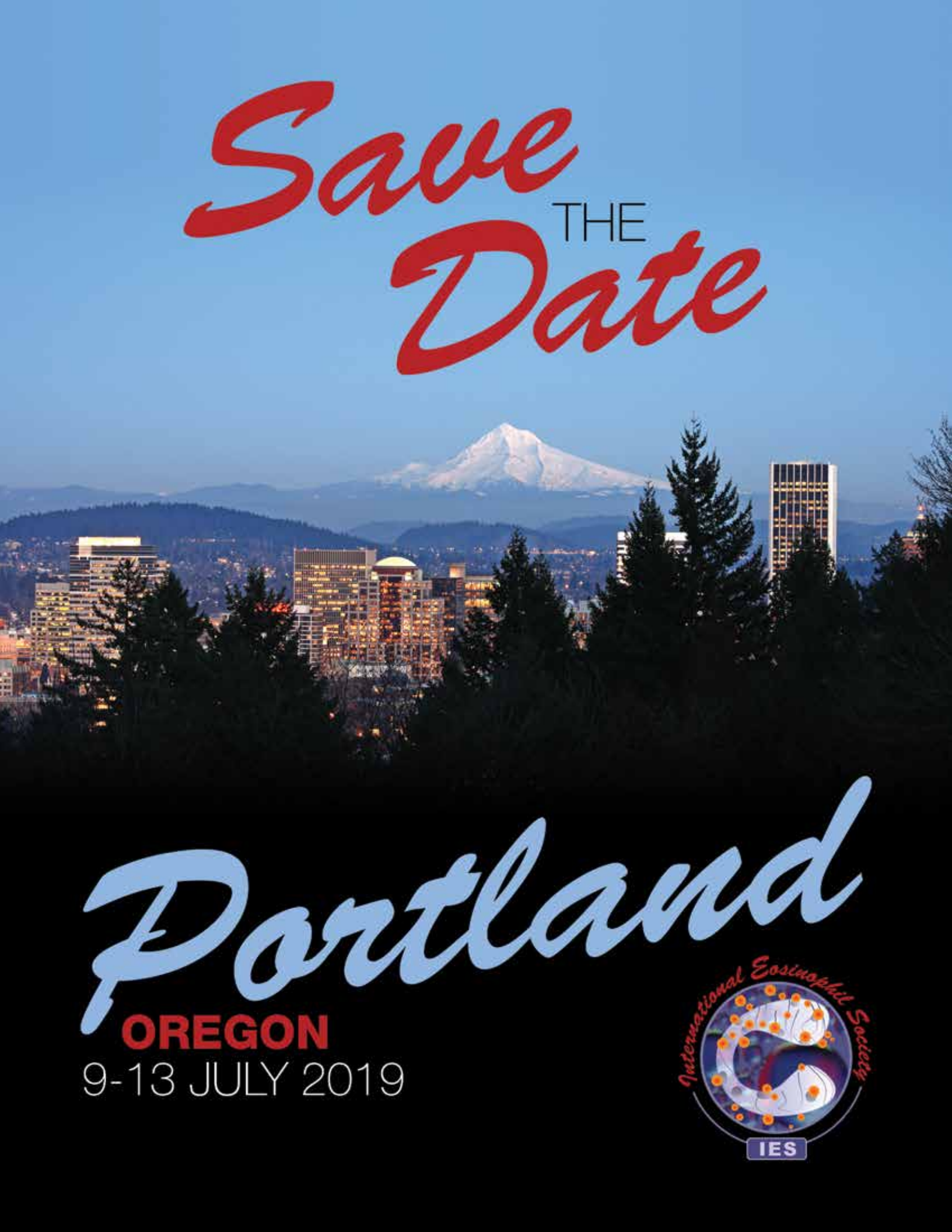
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