International Eosinophil Society, Inc.

Final Program

Board of Directors 2013-2015

President
Hans-Uwe Simon, MD, PhD
University of Bern
Bern, Switzerland

President-Elect
Peter Weller, MD
Harvard University
Boston, Massachusetts
United States

Immediate Past-President
Amy D. Klion, MD
National Institutes of Health
Baltimore, Maryland
United States

Secretary-Treasurer
Hirohito Kita, MD
Mayo Clinic Rochester
Rochester, Minnesota
United States

Scientific Program Director
Bruce S. Bochner, MD
Northwestern University
Chicago, Illinois
United States

Trainee Board Member
Neda Farahi, BSc, PhD
University of Cambridge
Cambridge, United Kingdom

At-Large Directors
Patricia T. Bozza, MD, PhD
Instituto Oswaldo Cruz
Rio de Janeiro, Brazil

Simon P. Hogan, PhD
Cincinnati Children's Hospital
Cincinnati, Ohio
United States

Leo Koenderman, PhD
University Medical Center Utrecht
Utrecht, Netherlands

James J. Lee, PhD
Mayo Clinic Arizona
Scottsdale, Arizona
United States

Kenji Matsumoto, MD, PhD
National Research Institute
Tokyo, Japan

Parameswaran Nair, MD
McMaster University
Hamilton, Ontario
Canada

Marc Rothenberg, MD, PhD
Cincinnati Children's Hospital
Cincinnati, Ohio
United States

Florence Roufosse, MD, PhD
Hospital Erasme (ULB)
Brussels, Belgium

Lisa A. Spencer, PhD
Beth Israel Deaconess Medical Center
Boston, Massachusetts
United States

Andrew Wardlaw, MD
Glenfield Hospital
Leicester, United Kingdom

Table of Contents

Welcome

Welcome fellow eosinomaniacs! After years of planning, we are excited to host you in Chicago for the 9th Biennial Symposium of the International Eosinophil Society, Inc. The 2015 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 7th in a series of clinically oriented workshops. The morning session, organized by Marc Rothenberg and Glenn Furuta, will focus on eosinophilic gastrointestinal diseases, and the afternoon session, assembled 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 Bill Busse and our second annual Eosinophil Jeopardy contest. Sessions beginning Wednesday morning and continuing until midday Saturday, 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 100 submitted abstracts. New to the meeting on Thursday morning is a breakfast symposium, presented by the World Allergy Organization. There will be plenty of opportunities for discussion during the three poster sessions, and prizes will be given to trainees at the Friday night Awards Dinner for best posters and oral presentations.

Of course, there will be free time each afternoon and Thursday evening for exploration of Chicago, with its world-class museums, parks, beaches, music, food, shopping and architecture under what we hope will be warm sunny skies. Did you know that the Art Institute of Chicago, just a 25 minute walk from the hotel, was voted the #1 museum in the world?

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, Ashley Salmon 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!

Hans-Uwe Simon
President

Bruce Bochner
Scientific Program Director

Acknowledgements

IES thanks the following partners for their support of the 9th Biennial Symposium

Gold Supporters

MedImmune/AstraZeneca

Teva Respiratory

Silver Supporters

IMMUNE Pharmaceuticals

Receptos

Roche

Takeda

Bronze Supporters

Allakos

Apfed

CURED

Diagnostics Development

Knopp Biosciences

TIGER

Additional Support

Novartis Institutes for BioMedical Research, Inc.

Society Sponsors

American Academy of Allergy, Asthma & Immunology

European Academy of Allergy and Clinical Immunology

Speaker Travel Support

Ku Leuven

VIB

Grant Support

National Institutes of Health

Funding for this conference was made possible [in part] by 1R13AI118098-01 from the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

General Information

Coffee Breaks

Coffee breaks are included in the registration fee for attendees and will be served daily. Coffee will be served outside of the Sauganash Grand Ballroom. Please check the Scientific Program for exact times.

Education Sessions

All education sessions will take place in the Sauganash Grand Ballroom East located on the 14th floor of the Holiday Inn Mart Plaza.

Meals

Continental breakfast will be available each morning prior to the first session. Breakfast will be served outside the Sauganash Grand Ballroom.

Poster Sessions

All poster sessions will take place in the Sauganash Grand Ballroom West. An assortment of beverage and snacks 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 Sauganash Grand Ballroom on the 14th floor of the Holiday Inn Mart Plaza on all days of the Symposium.

Hours:

Tuesday, 14 July	07:00-20:00
Wednesday, 15 July	07:30-13:00
	15:00-18:00
Thursday, 16 July	06:30-13:00
	15:00-18:30
Friday, 17 July	07:30-13:00
	15:30-18:30
Saturday, 18 July	07:30-13:00

Social Events

Welcome Reception and Dinner
Tuesday, 14 July, 17:30-21:00

The welcome reception will be held at the Holiday Inn Mart Plaza in the LaSalle room located on the 15th floor. Refreshments and dinner will be provided.

Chicago River Architectural Boat Ride and Pizza Party
Wednesday, 15 July, 19:15-21:45

The boat ride will depart at 19:15 from the Trump Towers boat dock located on the lower level of Rush Street and Kinsie Street behind the Trump Towers hotel.

Walking directions from Holiday Inn: Exit Merchandise Mart at Kinsie and Wells Streets, continue on Kinsie heading east until Rush Street, go south (right) on Rush Street towards river.

Awards Dinner
Friday, 17 July, 19:30-21:00

The awards dinner will be held at the Holiday Inn Mart Plaza in the Wolf Point Ballroom located on the 15th floor. Refreshments and dinner will be provided.

Pre-Conference Workshop Agenda

Tuesday, 14 July 2015

Consortium of Eosinophilic Gastrointestinal Disease Researchers (CEGIR) Symposium

8:00-8:20

Introduction to program and NIH U54 rare disease clinical disease research network
Glenn Furuta, United States and Marc Rothenberg, United States

8:20-8:50

Diagnostic criteria and related breakthroughs in EoE
Margaret Collins, United States

8:50-9:20

Pathophysiology of EoE
Marc Rothenberg, United States

9:20-9:50

Cellular etiology of tissue remodeling and management of strictures in EoE
Seema Aceves, United States

9:50-10:15

Coffee break

10:15-10:45

Comparing EoE vs EG and vs PPI-REE
Nirmala Gonsalves, United States

10:45-11:15

Validated monitoring and treating EoE
Evan Dellon, United States

11:15-11:45

Biological therapy and other new treatment approaches for EoE
Glenn Furuta, United States

12:00-13:00—Lunch (provided)

Personalized medicine: Novel biologic therapies for rare eosinophilic disorders

13:00-13:20

Opening remarks and introductions
Amy Klion, NIH, United States

I. Predictors of response to targeted therapies

Moderators: Patty Fulkerson, United States and Peter Weller, United States

13:20-13:40

Clinical features
Michael Wechsler, United States

13:40-14:00

Biomarkers
Paneez Khoury, United States

Pre-Conference Workshop Agenda

Tuesday, 14 July 2015

14:00-14:20

Molecular signatures
Marc Rothenberg, United States

II. An industry perspective

Moderator: Bruce Bochner, United States

14:20-14:30

Mepolizumab
Hector Ortega, Glaxo Smith Kline

14:30-14:40

Reslizumab
James Zangrilli, Teva

14:40-14:50

Benralizumab
Roland Kolbeck, MedImmune

14:50-15:00

Lebrikizumab
Joe Arron, Genentech

15:00-15:20

Coffee break

15:20-16:00

Panel discussion
Joe Arron, Genentech; Roland Kolbeck, MedImmune; Hector Ortega, Glaxo Smith Kline; Nenad Tomasevic, Allakos; James Zangrilli, Teva and Celia Zinger, Immune Pharmaceuticals

III. The patient perspective

16:00-16:20

Personal choice in the selection of therapy
Kathleen Sable, APFED and Ellyn Kodroff, CURED

IIII. A regulatory perspective

Moderator: Florence Roufosse, Belgium

16:20-17:00

Gaps in our understanding of predictors of response to therapy/research needs

Scientific Program

Tuesday, 14 July 2015

17:30-18:00

Welcome
Hans-Uwe Simon, Switzerland and Bruce Bochner, United States

Welcome lecture: The eosinophil and allergic inflammation: Out of the woods, almost.
William Busse, United States

18:00-21:00

Welcome Reception and dinner – Holiday Inn Mart Plaza, LaSalle room

20:00-21:00

2nd Annual Eosinophil Jeopardy – A quiz for eomaniacs
Bruce Bochner, United States

Wednesday, 15 July 2015

Eosinophil lifespan from start to finish

Session 1: Eosinophils and hematopoiesis

Chairs: Florence Roufosse, Belgium and Fei Li Kuang, United States

8:30-9:00

State-of-the-art: How to make an eosinophil
Steven Ackerman, United States

9:00-9:30

Cutting edge: When eosinophilopoiesis goes wrong
Jan Cools, Belgium

9:30-9:45

Abstract speaker: The role of Trib1 in eosinophil development
Ethan Mack, United States

9:45-10:00

Abstract speaker: The transcription factor XBP1 is selectively required for eosinophil differentiation
Sarah Bettigole, United States

10:00-10:30

Cutting edge: IL-33 and its role in eosinophil hematopoiesis
Paul Bryce, United States

10:30-11:00

Coffee break

Wednesday, 15 July 2015

Session 2: Eosinophil migration, accumulation and activation

Chairs: Joan Cook-Mills, United States and Neda Farahi, United Kingdom

11:00-11:30

State-of-the-art: Eosinophil trafficking
P. Sriramarao, United States

11:30-12:00

Cutting edge: Regulation of eosinophil accumulation
Ariel Munitz, Israel

12:00-12:15

Abstract speaker: Eosinophil mediators contribute to lung remodeling and dysfunction in a model of severe asthma
Elizabeth Jacobsen, United States

12:15-12:30

Abstract speaker: Pin1 is required for eosinophil migration mediated by G protein-coupled receptor EBI2
Zhong-Jian Shen, United States

12:30-13:00

Cutting edge: Eosinophil activation and mediator release
Peter Weller, United States

13:00-15:30—Lunch (on your own)

Session 3: Eosinophil survival and death

Chairs: Mats Johansson, United States and Jeremy O'Sullivan, United States

15:30-16:00

State-of-the-art: Eosinophil survival
Francesca Levi-Schaffer, Israel

16:00-16:30

Cutting edge: Eosinophil death pathways
Hans-Uwe Simon, Switzerland

16:30-16:45

Abstract speaker: Specific subsets of kinases mediate Siglec-8 engagement-induced ROS production and apoptosis in eosinophils
Daniela Janevska, United States

16:45-17:00

Abstract speaker: Dissecting glucocorticoid (GC) pathways regulating eosinophil viability in cytokine-activated eosinophils: Role of protein phoshatase 5 on suppression of GC receptor and ASK1 phosphorylation
Konrad Pazdrak, United States

Wednesday, 15 July 2015

17:00-17:30

Dr. Redwan Moqbel Memorial Lecture

Abstract speaker: IL-1ß in eosinophil-mediated gastrointestinal homeostasis and immunoglobulin A production
YunJae Jung, Korea

17:30-18:45—Poster Session 1 (Sauganash West room)

Eosinophils and hematopoiesis

1 Peripheral B Cells and eosinophils are positively correlated in untreated human subjects with eosinophilia
Fei Li Kuang

2 Identification of the transcription factor repertoire during homeostaticeosinophilopoiesis
Patricia Fulkerson

3 TNF mediates hematopoesis of benifical eosinophils three days after ozone
Sarah Wicher

4 Eosinophil progenitor mobilization in active eosinophilic esophagitis
David Morris

5 Age and gender demographic analysis of cases referred for investigation of FIP1L1-PDGFRA positive chronic eosinophilic leukemia in Australia
Alberto Catalano

6 Maternal supplementation of allergic female mice with gamma-tocopherol increases the development of select dendritic cell subsets and allergic lung inflammation in neonates
Joan Cook-Mills

Eosinophil migration, accumulation and activation

7 Exosomes secretion by eosinophils: A possible role in asthma pathogenesis
Victoria Del Pozo

9 Osteopontin binds and modulate functions of eosinophil-recruiting chemokines
Anele Gela

10 Human eosinophils release extracellular DNA traps in response to *aspergillus fumigatus*
Josiane Neves

11 Cannabinoid receptor 2 augments human eosinophil responsiveness via G GAQ/MEK/rock signaling
Robert Frei

12 Eosinophil associated CD48: Regulation and the role of its soluble form in staphylococcus aureus enterotoxin B induced eosinophil activation
Roopesh Gangwar

13 Adipose tissue of CCR2 deficient mice display increased eosinophil accumulation, type 2 cytokine expression, and alternative macrophage polarization
W. Reid Bolus

14 Prolonged RSK and RPS6 phosphorylations by IL-3 increases translation in human eosinophils
Stephane Esnault

15 Alterations in the polarized morphology of interleukin-5-stimulated eosinophils upon adhesion to periostin, and periostin clearance involving ADAM8
Mats Johansson

16 Comparative proteomics of unactivated and activated peripheral blood eosinophils
Mats Johansson

17 Galectin-1: Role in regulation of eosinophilia and allergic airway inflammation
Xiao Ge

18 SPECT/CT to quantify eosinophil migration into the lungs
Chrystalla Loutsios

19 A novel protease, PRSS33 (serine protease 33; EOS), is specifically and constitutively expressed in human eosinophils
Kenji Matsumoto

20 Leukotriene B4: Underappreciated regulator of human eosinophils
Nicholas Flamand

21 Metabolism of exogenous arachidonoyl-ethanolamide and 2 arachidonoyl-glycerol by human eosinophils
Nicholas Flamand

22 Eosinophils drive cardiac remodeling and development of heart failure
Daniela Čiháková

23 A comparative analysis of eosinophil cell surface markers in eosinophilic disorders
Fanny Legrand

24 The availability and utility of eosinophil-specific reagents and mouse models from Lee Laboratories
James Lee

25 Eosinophil trafficking to the heart in eosinophilic myocardits is dependent on CCR3
Nicola Diny

26 Notch signaling is required for the priming-induced enhanced migration of human eosinophils *in vitro*, and recruitment of mouse eosinophils into allergiclungs *in vivo*
Lisa Spencer

27 Tumor eosinophil infiltration and survival of colorectal cancer patients
Anna Prizment

28 Identification of the transcription factor aiolos as a novel regulator of eosinophil trafficking
Patricia Fulkerson

29 Differential localization of STAT3α and STAT3β in acutely activated eosinophils
Keren Turton

Eosinophil survival and death

30 Ligand-mediated Siglec-8 internalization in eosinophils is influenced by the actin cytoskeleton, tyrosine kinases, dynamin, and sialylated *cis* ligands
Jeremy O'Sullivan

31 D-type prostanoid receptor signaling promotes survival by inhibition of the intrinsic apoptosis pathway and activates related gene regulation elements
Miriam Peinhaupt

32 Eosinophils undergo cyclophilin D-dependent regulated necrosis
Nives Zimmermann

33 Distinct modes of reactive oxygen species generation in eosinophil cell death induced by Siglec-8/IL-5 co-stimulation and extracellular DNA trap cell death
Gen Kano

19:15-21:45—Chicago river architectural boat ride with pizza party and Navy Pier fireworks

Walking directions located on page 5.

Thursday, 16 July 2015

Breakfast Symposium, presented by the World Allergy Organization
**Please note that breakfast will only be provided for those attending the breakfast session (all attendees welcome)*

7:00-8:15

Cytokine Families in the Activation of Eosinophils in Severe Asthma
Lanny Rosenwasser, United States

Phenotypes of Severe Asthma: Role of Eosinophils
Eugene Bleecker, United States

Eosinophils causing trouble

Session 4: Eosinophilic GI disease

Chairs: Nirmala Gonsalves, United States and Netali Ben Baruch Morgenstern, Israel

8:30-9:00

State-of-the-art: Pathomechanisms of eosinophilic esophagitis
Marc Rothenberg, United States

9:00-9:30

Cutting edge: Characterization of pathogenic Th2 cells in EGID
Calman Prussin, United States

9:30-9:45

Paired immunoglobulin-like receptor B inhibits eosinophil accumulation and activation in eosinophilic esophagitis
Netali Ben-Baruch-Morgenstern

9:45-10:00

Abstract speaker: Resident intestinal eosinophils acquire luminal antigen through low affinity Fc gamma receptors
Lisa Spencer, United States

10:00-10:30

Cutting edge: Mechanisms of tissue remodeling in EoE
Seema Aceves, United States

10:30-11:00

Coffee break

Session 5: Eosinophilic airways disease

Chairs: Allison Fryer, United States and Manali Mukherjee, Canada

11:00-11:30

State-of-the-art: Role of eosinophils in chronic rhinosinusitis
Robert Schleimer, United States

11:30-12:00

Cutting edge: Eosinophils, asthma and the microbiome
Andrew Wardlaw, United Kingdom

12:00-12:15

Abstract speaker: Oncostatin M is elevated in patients with eosinophilic mucosal disease and promotes epithelial barrier dysfunction
Kathryn Pothoven, United States

12:15-12:30

Abstract speaker: Eosinophils regulate airway nerve substance P expression
Katie Lebold, United States

12:30-13:00

Cutting edge: Pathophysiology and management of EGPA
Michael Wechsler, United States

13:00-15:30—Lunch (on your own)

Session 6: Eosinophils and related players in skin disease

Chairs: Hirohito Kita, United States and Marzyeh Amini, Netherlands

15:30-16:00

State-of-the-art: Eosinophils and atopic dermatitis
Sabina Islam, United States

16:00-16:30

Cutting edge: IL-13, eosinophils and itch
Tao Zheng, United States

16:30-16:45

Abstract speaker: Eosinophils stimulate sensory neuroplasticity
Quinn Roth-Carter, United States

16:45-17:00

Abstract speaker: CD3-CD4+ T cell-associated urticaria and angioedema without eosinophilia: A new benign lymphoproliferative disorder
Florence Roufosse, Belgium

17:00-17:30

Cutting edge: Atopic dermatitis and immunodeficiency
Joshua Milner, United States

17:30-18:45—Poster Session 2 (Sauganash West room)

Eosinophilic GI disease

35 Active eosinophilic esophagitis is characterized by epithelial barrier defects and eosinophil extracellular trap formation
Dagmar Simon

36 Eosinophil regulates transcriptional expression of genes involved in various physiologic responses in the small intestine
YunJae Jung

37 Significance of food skin prick testing in adult eosinophilic esophagitis patients
Ashleigh Olson

38 A key requirement for CD300LF regulating innate immune responses of eosinophils in colitis
Itay Moshkovits

39 Eosinophils from eosinophilic esophagitis patients express FOXP3 and use Galectin-10 to suppress T cells
Jennie Andersson

40 Age-dependent differences in the molecular patterns of eosinophils from eosinophilic esophagitis patients and healthy persons
Christine Wennerås

41 Esophageal immunoglobulin levels in eosinophilic esophagitis esophageal tissue
Melissa Mingler

42 Eosinophil granule proteins in stool: A potential non-invasive biomarker of eosinophil infiltration in gut tissue
Michelle Makiya

43 IL-33 is selectively expressed by esophageal epithelial progenitor cells during allergic inflammation
Jared Travers

44 Claudin-7 dysregulation in pediatric eosinophilic esophagitis: A role for TGF-β1 in esophageal epithelial barrier dysfunction
Joanne Masterson

45 CDH26 is an integrin-binding immunomodulator involved in allergic inflammation
Julie Caldwell

Eosinophilic airways disease

46 Alveolar macrophages initiate inflammatory responses to lactobacillus plantarum in the mouse airway
Tyler Rice

47 Extracellular DNA traps in bronchial secretion from allergic bronchopulmonary aspergillosis
Shigeharu Ueki

48 Correlation between CCL26 production by human bronchial epithelial cells and airway eosinophils: Involvement in patients with severe eosinophilic asthma
Marie-Chantal Larose

49 Type 2 cytokine-producing innate lymphoid cells (ILC2) in bronchoalveolar lavage (BAL) and blood from human asthma and their context-dependent response to glucocorticoids
Rafeul Alam

50 Mechanisms involved in the expression of CCL26 by bronchial epithelial cells: Importance in asthma and its severity
Marie-Chantal Larose

51 A novel mechanism for virus-induced asthma exacerbation: Immune memory to virus can induce airway hyperreactivity in allergic animals
Darryl Adamko

52 Eosinophils display damaging and protective properties upon viral exposure; its relevance in virus-induced loss of asthma control
Yanaika Sabogal Piñeros

Thursday, 16 July 2015

53 Differential activation of airway eosinophils promotes IL-13 induced pulmonary responses following allergen provocation
Elizabeth Jacobsen

54 Local autoimmunity in severe eosinophilic asthma
Manali Mukherjee

55 Novel scoring system and algorithm for classifying eosinophilic chronic rhinosinusitis: The JESREC study
Takahiro Tokunaga

Eosinophils and related players in skin disease

56 Eosinophil degranulation and the release of eosinophil peroxidase contributes to the induced inflammation occurring in mice following skin exposure to TMA
Huijun Luo

Dinner (on your own)

Friday, 17 July 2015

Eosinophils wanted: dead or alive – translation of basic mechanisms to therapeutics

Session 7: Eosinophils in innate and adaptive immunity (part 1)

Chairs: Lisa Spencer, United States and Fabiola Cortinas-Elizondo, Switzerland

8:30-9:00

State-of-the-art: ILC2s and eosinophilic inflammation
Taylor Doherty, United States

9:00-9:30

Cutting edge: Deleting eosinophils: lessons learned
James Lee, United States

9:30-9:45

Abstract speaker: Immune regulatory siglec ligands and their upregulation in inflamed human airways
Steve Fernandes, United States

9:45-10:00

Abstract speaker: Increased numbers of LL-5+IL-13+ group 2 innate lymphoid cells in sputum of steroid dependent severe asthmatics with persisitent airway eosinophilia
Steven Smith, Canada

10:00-10:30

Cutting edge: Glycobiology and eosinophilic inflammation
Bruce Bochner, United States

10:30-11:00

Coffee break

Friday, 17 July 2015

Session 8: Eosinophils as therapeutic targets (part 1)

Chairs: Parameswaran Nair, Canada and Sophie Fillon, United States

11:00-11:30

State-of-the-art: Current treatment options and unmet needs for EGID
Ikuo Hirano, United States

11:30-12:00

Cutting edge: Optimizing treatment options for HES including Workshop recommendations
Amy Klion, United States

12:00-12:15

Abstract speaker: Claudin-1 dysregulation in eosinophilic esophagitis: A role for HIF-1A in esophageal epithelial barrier dysfunction
Joanne Masterson, United States

12:15-12:30

Abstract speaker: MicroRNA-155 regulates airway group 2 innate lymphoid cells (ILC2) in murine models of allergic airway inflammation
Kristina Johansson, Sweden

12:30-13:00

Cutting edge: miRNAs in eosinophil biology and lung inflammation
Ming Yang, Australia

13:00-15:30—Lunch (on your own)

15:30-16:00—IES Business Meeting (All are Welcome)

Session 9: IES award lectures

Chairs: Hans-Uwe Simon, Switzerland

16:00-16:30

Gleich Award
Jesse Nussbaum, United States

16:30-17:30

Ehrlich Lectureship
Gerald Gleich, United States

17:30-18:45—Poster Session 3 (Sauganash West room)

Eosinophils in innate and adaptive immunity (part 1)

57 Innate immune system crosstalk: Eosinophils mediate immune modulation of dendritic cells
Fabiola Cortinas-Elizondo

58 TIM-4 expressed on eosinophils from lung- and gut-draining lymph nodes costimulates T cell expansion
Haibin Wang

59 Allergen-induced increases in IL-25 and IL-25 receptor expression in mild asthmatics: Mechanism for promoting lung homing of mature and lineage-committed progenitors of eosinophils
Steven Smith

60 Predicting bladder cancer patient response to bacillus calmette-guérin using a novel diagnostic strategy assessing the pre-treatment tumor immune microenvironment
James Lee

Eosinophils as therapeutic targets (part 1)

61 A proof-of-concept study with a novel anti-IL-13 monoclonal antibody (RPC4046) in adults with eosinophilic esophagitis: Design and outcome measures of the HEROES study
Ikuo Hirano

62 A multiple-food elimination diet is effective for the treatment of eosinophilic gastroenteritis
Yoshiyuki Yamada

63 A curious case of cyclic vomiting with eosinophilia
Santhosh Kumar

64 Impact of treatment on the esophageal microbiota and bacterial receptor expression in eosinophilic esophagitis
Sophie Fillon

65 Dexpramipexole for the treatment of chronic rhinosinusitis with nasal polyps: Preliminary findings in an open-label proof of concept trial
Michael Bozik

66 Source of interleukin-5 in familial eosinophilia
Senbagavalli Prakash Babu

Eosinophils in innate and adaptive immunity (part 2)

67 Eosinophils regulate intestinal B- and T-cell responses following infection with the nematode *heligmosomoides polygyrus*
Julia Strandmark

68 Eosinophils protect alveolar macrophages from acute respiratory virus infection *in vivo*
Kimberly Dyer

69 Eosinophil as a universal determinant underlying common complex diseases and quantitative intermediate traits
Marzyeh Amini

70 Detection of human eosinophil extracellular vesicles by nanoscale flow cytometry
Praveen Akuthota

71 Eosinophils promote antiviral host defenses against influenza a virus infection
Amali Samarasinghe

Eosinophils as therapeutic targets (part 2)

72 Variation at the 17q21 asthma locus is associated with FeNO levels, peripheral blood eosinophil counts, and eosinophil activation in humans
Elizabeth Schwantes

73 Regulation of asthma-susceptibility ORMDL3 gene expression in eosinophils and lung epithelial cells
Sung Ha

74 Minimally invasive measurement of inflammation in eosinophilic esophagitis using an eosinophil peroxidase (EPO) brush assay
Hedieh Saffari

Friday, 17 July 2015

75 Is periostin a good biomarker for asthma?
Marie-Chantal Larose

76 Complete reversal of CNS manifestations and sustained molecular response for 6 years in chronic eosinophilic leukaemia (CEL) FIP1L1-PDGFRA+ after imatinib therapy
Finella Brito-Babapulle

77 Zinc oxide nanoparticles can alter the biology of human eosinophils
Luis Silva

78 Stability of blood eosinophil count and FeNO in human subjects
Paul Fichtinger

79 Identification of degranulation patterns and vesicular transport of major basic protein in eosinophils triggered by the experimental schistosoma mansoni infection
Rossana Melo

80 Increased detection of esophageal inflammation but not remodeling by endoscopy compared with radiologic imaging in adults with eosinophilic esophagitis
Matthew Nelson

81 Development and validation of an easy to use assay for the assessment of eosinophil peroxidase in sputum as a sensitive/reproducible biomarker for patient diagnosis
Sergei Ochkur

82 Mechanisms of glucocorticoid resistance in HES
Kindra Stokes

19:30-21:00—Awards Dinner

Saturday, 18 July 2015

Eosinophils wanted: dead or alive – translation of basic mechanisms to therapeutics

Session 10: Eosinophils in innate and adaptive immunity (part 2)

Chairs: Patricia Bozza, Brazil and Julia Strandmark, Germany

8:30-9:00

State-of-the-Art: Eosinophils in Th2 mediated lung inflammation
Clare Lloyd, United Kingdom

9:00-9:30

Cutting edge: Eosinophils, mast cells and food allergy
Simon Hogan, United States

9:30-9:45

Abstract speaker: Eosinophils Promote Colorectal Cancer through Expression of S100a8 and S100a9
Hadar Reichman, Israel

9:45-10:00

Abstract speaker: Similar phenotypic changes on eosinophils in different lung compartments during normal postnatal lung development and allergic inflammation suggest their homeostatic function in the airway mucosa
Sergejs Berdnikovs, United States

Saturday, 18 July 2015

10:00-10:30

Cutting edge: Eosinophil-parasite interactions
Rachel A. Lawrence, United Kingdom

10:30-11:00

Coffee break

Session 11: Eosinophils as therapeutic targets (part 2)

Chairs: Raphael Alam, United States and Sung Gil Ha, United States

11:00-11:30

State-of-the-art: Biologics for asthma
Gail Gauvreau, Canada

11:30-12:00

Cutting edge: Novel eosinophil therapeutic targets
Paneez Khoury, United States

12:00-12:15

Abstract speaker: Efficacy, safety, and patient-reported outcomes with reslizumab in patients with asthma and elevated blood eosinophils: a randomized phase 3 study
James Zangrilli, United States

12:15-12:30

Abstract speaker: Development Of A Novel Peptide Nanoparticle-Based Antagonist Of Human CCR3-Mediated Eosinophil Migration In Allergic Inflammation
Kimberly G. Laffey, United States

12:30-13:00

Cutting edge: Treatment of eosinophil-related skin disorders
Dagmar Simon, Switzerland

Acknowledgements and adjournment
Hans-Uwe Simon, United States; Bruce Bochner, United States

Gleich Award

Dr. Jesse Nussbaum will receive the third Gerald J. Gleich prize to be awarded at the 9th Biennial Symposium of the International Eosinophil Society, Inc. in Chicago, IL USA. 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.

Dr. Jesse Nussbaum received a medical degree at Duke University School of Medicine and completed an internal medicine residency at Yale-New Haven Hospital and Infectious Disease fellowship at UCSF in 2010. He practices clinical Infectious Diseases at UCSF and also sees patients at the UCSF HIV/AIDS clinic. His research involves using mouse models to define signals that mediate host-helminth interactions, with the goal of modulating these pathways to treat human disease. He is currently supported by a K08 Career Development grant and is recipient of the 2015 ASCI Council Young Physician-Scientist Award.

Recipient of the 2015 Gleich Award

The Gerald J. Gleich Award
International Eosinophil Society, Inc.
2015

Jesse Nussbaum, MD

In recognition of the most intriguing, high impact finding related to eosinophil biology published in the years 2013-2015.

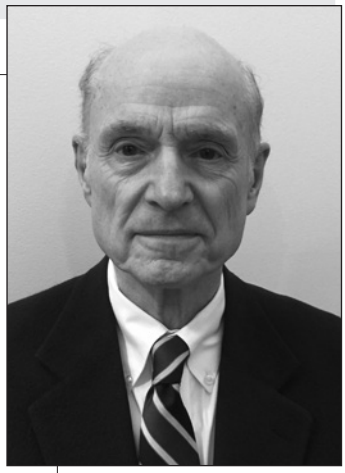
International Eosinophil Society
IES

Ehrlich Lectureship

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

Dr. Gerald J. Gleich was born in Escanaba, Michigan and received his degree in Medicine from the University of Michigan. He obtained Internal Medicine residency training at Philadelphia General Hospital and Jackson Memorial Hospital and was a flight surgeon in the United States Air Force. He received postdoctoral training at the University of Rochester and then established a research laboratory for allergic diseases at the Mayo Clinic and Foundation. At Mayo, he was Professor of Medicine and Immunology, Chair of the Department of Immunology, Distinguished Investigator of the Mayo Foundation, and the George M. Eisenberg Professor of Medicine and Immunology. Presently, he is Professor of Dermatology and Medicine at the University of Utah. Dr. Gleich has had a life-long professional commitment to understanding the eosinophil with a focus on its distinctive granules. He and his colleagues have isolated, characterized, identified the cDNAs and the genes and established assays for measurement and localization of all of the principal granule proteins. These efforts lead to the recognition that eosinophil degranulation with release of cytotoxic and cytostimulatory cationic proteins into tissues is characteristic of eosinophil-associated diseases and to recognition of several novel syndromes. Dr. Gleich has served on many committees and editorial boards, including Chair of the WHO Subcommittee on Standardization of Allergens, the editorial board of the *Journal of Allergy and Clinical Immunology*, the editorial Board of the *Journal of Immunology*, Member and Chair of the Board of Scientific Counselors, NIAID, Member and Chair of the Immunological Sciences Study Section NIH and Chair of the Data and Safety Monitoring Board of the NIAID. Dr. Gleich has received numerous awards and honors, including memberships in Phi Beta Kappa, Phi Kappa Phi, Alpha Omega Alpha and Sigma Xi, the American College of Physicians (Fellow), the American Society for Clinical Investigation, the American Association of Immunologists, the Collegium Internationale Allergologicum, the Association of American Physicians; named lectureships including the John M. Sheldon Memorial Lecturer of the American Academy of Allergy, Asthma and Immunology 1976, 1982 and 1988, the Stoll-Stunkard Lecturer American Society of Parasitologists, Distinguished Lecturer in Medical Sciences Mayo Clinic and Foundation; and awards including the Landmark in Allergy Award Recipient, Fellowship award of the American Association for the Advancement of Science, Honorable Membership in the Pharmacia Allergy Research Foundation and Original Member, Highly Cited Researchers database, ISI Thomson Scientific. Lastly, Dr. Gleich has contributed over 600 articles to scientific literature.

Recipient of the 2015 Ehrlich Lectureship



Paul Ehrlich Lectureship
International Eosinophil Society, Inc.
2015

Gerald J. Gleich, 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.

Service Award

2015 International Eosinophil Society Service Award to Amy D. Klion, MD

The International Eosinophil Society, Inc., its leaders and members present to Dr. Amy D. Klion the distinguished Service Award both for her cardinal leadership in helping found and advance the International Eosinophil Society, Inc. and for her career-long contributions to innovative studies of the immunobiology of eosinophils.

Amy D. Klion, MD earned her BA from Princeton University in 1981 and her MD from New York University School of Medicine in 1985. After completing a residency in Internal Medicine at Johns Hopkins University in 1988, she was a postdoctoral fellow in the Laboratory of Parasitic Diseases (LPD) at the NIH. She completed her fellowship in Infectious Diseases at the University of Iowa Hospitals and Clinics and was appointed an assistant professor in the Division of Infectious Diseases prior to returning to the LPD in 1997, where she is currently a tenured Clinical Investigator and head of the Human Eosinophil Section. In the early stages of her career, Dr. Klion focused primarily on questions related to the diagnosis, treatment and pathogenesis of the filarial parasite, Loa loa. Since returning to the LPD, however, her research program has expanded to include basic and translational research related to the role of the eosinophil and eosinophil activation in disease pathogenesis, with a strong emphasis on rare hypereosinophilic syndromes. A member of the International Eosinophil Society since 1999, Dr. Klion has served on the Executive Board and as Past President. Friendships and collaborations formed through the IES have enriched her life immeasurably, both in and outside of the laboratory.

Recipient of the 2015 Service Award

Service Award
International Eosinophil Society, Inc.
2015

Amy D. Klion, MD

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

International Eosinophil Society
IES

In Memoriam

Redwan Moqbel, PhD

Born in a border town on the Iraq/Iran border (14 August 1947), Redwan's family history is linked with the earliest days of the Baha'i Faith. Redwan served the Baha'i community in the UK and Canada in volunteer capacities, including as a member of the national governing council of the Baha'i community of the United Kingdom for 13 years.

Redwan was a speaker of rare eloquence, clarity and depth whose spiritual beliefs were firmly anchored in Baha'u'llah's writings and whose abundant humour was never at the expense of others. His life-long focus was on creating unity. He loved everyone but particularly youth whom he mentored on three continents. In confirmation of his efforts, Redwan received the Lieutenant Governor of Manitoba's Award for the Advancement of Interreligous Understanding in January 2013.

In 1976, Redwan obtained his PhD at the University of London, UK (LSH & TM). He became a faculty member there at the National Heart and Lung Institute in 1980. He was among the first to identify the immunological cell types that regulate asthma and allergy.

Recruited to the Department of Medicine, University of Alberta as a Professor in 1995, he served as the Director of the Pulmonary Research Group. There he received such prestigious awards as Alberta Heritage Medical Senior Scholar, Heritage Scientist and Heritage Senior Investigator.

In 2008, Redwan became Professor and Head of the Department of Immunology at the University of Manitoba, and Professor Emeritus at the University of Alberta. He was well recognized for his mentorship of young biomedical scientists, whom he encouraged to adopt "a noble goal."

An international authority on the immuno-molecular basis of asthmatic inflammation, in particular the role of eosinophils, Redwan's research garnered him numerous distinctions and awards. The International Eosinophil Society, of which he was a founding member, awarded him their highest honour, The Paul Ehrlich Award, named a mentoring award after him, and further honoured him with the prestigious Service Award in recognition of his "cardinal leadership" and innovative research.

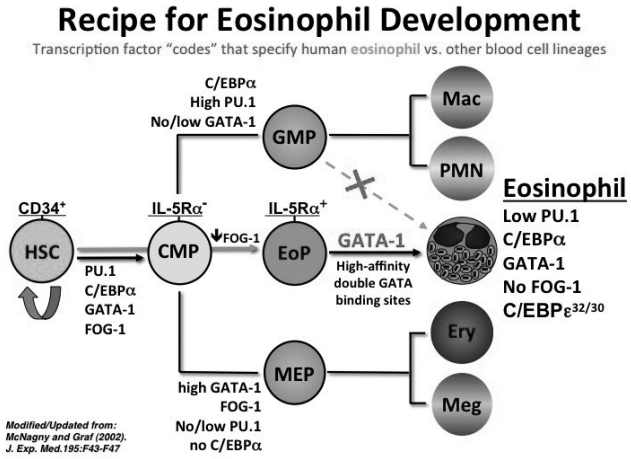
A recent example of his work as a champion reconciler was his role in organizing a scientific conference in which protagonists in the controversy over Lyme Disease came together in an atmosphere of mutual respect.

STATE-OF-THE-ART: HOW TO MAKE AN EOSINOPHIL – A DEFINITIVE (FAVORITE) RECIPE

Steven J. Ackerman, Kimberly G. Laffey and Jian Du.

Dept. of Biochemistry and Molecular Genetics, and Department of Medicine, University of Illinois at Chicago, Illinois.

Under homeostatic conditions, eosinophil development from a distinct lineage-committed hematopoietic progenitor cell population (EoP) is regulated at multiple levels that include intrinsic factors (combinatorial transcription factor networks, miRNAs, lncRNAs, signaling by the bone marrow microenvironment), while extrinsic factors (e.g. ILC2 and Th2-lymphocyte-derived growth factors and lineage-specific cytokines) largely control eosinophil progenitor cell expansion and terminal differentiation (for development of blood and tissue eosinophilia) in response to inflammation (e.g. allergen sensitization and challenge), infectious agents (e.g. helminth parasites), and tissue damage and repair processes. Regulation of hematopoietic stem cell lineage commitment to EoPs, their expansion, and terminal differentiation will be reviewed, including recipes for generation of eosinophils from CD34+/IL-5Rα EoPs and induced pluripotent stem cells (iPSCs).



Ingredients:

Cells	Transcription and Other Factors	Cytokines
CD34+ (IL-5Rα+) EoPs	C/EBPα (1 tsp)	SCF, GM-CSF
	PU.1 (1/4 tsp)	IL-3, IL-5
	GATA-1 (1 tsp, must be Fog-1 free)	
	(or GATA-2 if you don't have any GATA-1)	
	C/EBPε (1 tbs, 32kD or 30kD isoforms)	
	C/EBPε(1 tsp each 27kD/14kD isoforms)	
	Pinch of ID(2), dash of EGO (lncRNA)	

Directions:
Start with umbilical cord, bone marrow, or peripheral blood CD34+ cells (~1 x 10^6).
Season the GATA-1, IL-5Rα and eosinophil granule protein promoters/enhancers with high affinity double GATA sites.
Combine and add transcription factors (except C/EBPε repressor isoforms).
Add a dash of EGO.
Bake in SCF/IL-3 at 37°C for 2-3 days to expand.
Add more IL-3, and add IL-5.
Continue baking at 37°C for 1-2 weeks, adding more IL-5 to taste as needed.
Add ID-2 and C/EBPε repressors (1/2 tsp, 27/14kD isoforms) (promotes terminal differentiation),
Continue baking until fully developed (~ 1 week)
(**Note:** EoPs from dual MBP-1/EPX null mice are defective in granulogenesis and will not work)
Serves up to ~10 (x 106) well-differentiated eosinophils

Grant support: Supported by NIH R21HL118588, R01AI33043, FDA 1R01FD004086, and HOPE Pilot Grants from the American Partnership for Eosinophilic Disorders.

STATE-OF-THE-ART: EOSINOPHIL TRAFFICKING

P. Sriramarao, Xiao Na Ge, Sung Gil Ha, Yana Greenberg and Savita P. Rao

Laboratory of Allergy and Inflammation, University of Minnesota, St. Paul, MN, USA

Eosinophil adhesive interactions within inflamed blood vessels and the subsequent activation and degranulation of recruited cells in inflamed tissue(s) are important contributors to the pathogenesis of allergic inflammation. Understanding the mechanisms by which key cellular factors/mediators regulate eosinophil trafficking and their recruitment to targeted inflamed tissue is central to amelioration of allergic disease. Several cytokines including those elaborated by Th2 cells play a critical role in the regulation of adhesion molecule-mediated eosinophil rolling, adhesion and transmigration in vivo. Intravital microscopy studies of eosinophil trafficking in animal models including allergen-challenged gene-deficient mice have revealed that chemokines/chemoattractants such as eotaxin, C3a, C5a, serotonin, etc., and their receptors differentially induce eosinophil recruitment to sites of inflammation. In addition, the relative importance of L/E/P-selectin, PSGL, VCAM-1, ICAM-1 well as α4 and β2 integrins to eosinophil trafficking and onset of allergic inflammation has been delineated. More recently the role of intracellular proteins such as ORMDL3 and SWAP-70 as well as the participation of glycan binding lectins such as galectins and siglec(s) and cell surface-expressed carbohydrates (*N*- and *O*-glycans, heparan sulfate proteoglycans) in the regulation of eosinophil function and trafficking has been demonstrated. While most studies have focused on how eosinophils traffic to inflammatory sites where they function as end stage degranulating effector cells of innate immunity, recent studies suggest an additional immunomodulatory role for eosinophils including their ability to traffic to lymph nodes to activate adaptive immune cells. In this presentation, an overview of the regulation of eosinophil trafficking and recruitment during allergic inflammation will be discussed.

REGULATION OF EOSINOPHIL ACCUMULATION AND ACTIVATION BY CD300F

Ariel Munitz, PhD[a]

[a] Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, Tel-Aviv University, Ramat Aviv 69978, Israel.

Background: Accumulation of eosinophils in health and disease is a hallmark characteristic of mucosal immunity, type 2 inflammatory responses as well as tissue regeneration and metabolism. Over the past years we have been interested in the roles of immunoreceptor tyrosine-based inhibitory and activating receptors in multiple cells including eosinphils

Results: We establish that CD300f is distinctly expressed by eosinophil progenitors and that its expression is tightly regulated by IL-5. Furthermore, under baseline conditions CD300f is highly and distinctly expressed by colonic and adipose tissue eosinophils. Interestingly *Cd300f-/-* mice displayed elevated baseline tissue eosinophilia. *Cd300f* negatively regulated eotaxin-induced eosinophil responses including eosinophil chemotaxis, actin polymerization, calcium influx and ERK-1/2 phosphorylation. Blockade of CD300f-ligand interactions rendered wild type eosinophils hyper-chemotactic in-vitro and in-vivo in a model of allergic airway disease. Notably, suppression of cellular recruitment via CD300f was specific to eosinophils and eotaxin, since LTB4- and MIP-1α-induced eosinophil and neutrophil migration were not negatively regulated by CD300f. Strikingly and despite the finding that CD300f acted as a negative regulator of eosinophil chemotactic responses towards eotaxins, CD300f amplified IL-4- and IL-33-induced eosinophil activation by augmenting cellular signaling, mediator release and priming. Moreover, CD300f was required for innate immune pro-inflammatory signaling and mediator release from eosinophils. Indeed, dextran sodium sulfate (DSS)-treated *Cd300f-/-* mice exhibit attenuated disease in comparison to DSS-treated WT mice. Decreased disease activity in *Cd300f-/-* mice was accompanied with reduced inflammatory cell infiltration and nearly abolished production of pro-inflammatory cytokines. Eosinophil depletion experiments in *Cd300f-/-* mice revealed that decreased disease activity in DSS-treated *Cd300f-/-* mice was largely due to its expression in eosinophils.

Conclusions: These data provide new insights into the molecular mechanisms governing eosinophil accumulation and mediator release. These data may provide new therapeutic tools to target eosinophils in various eosinophil-associated diseases.

References: Moshkovits I, Shik D, Itan M, Karo-Atar D, Bernshtein B, Hershko AY, van Lookeren Campagne M, Munitz A. CMRF35-like molecule 1 (CLM-1) regulates eosinophil homeostasis by suppressing cellular chemotaxis. *Mucosal Immunol*. 2014;7:292-303.

Shik D, Moshkovits I, Karo-Atar D, Reichman H, Munitz A. Interleukin-33 requires CMRF35-like molecule-1 expression for induction of myeloid cell activation. *Allergy*. 2014;69:719-29.

Moshkovits I, Karo-Atar D, Itan M, Reichman H, Rozenberg P Morgenstern-Ben-Baruch N, Shik D, Ejarque-Ortiz A, Hershko AY, Tian L, Coligan JE, Sayos J, Munitz A. CD300f associates with IL-4 receptor α and amplifies IL-4-induced immune cell responses. *PNAS, Accepted*

Grant support: US-Israel Binational Science Foundation (grant nos. 2009222 and 2011244), the Israel Science Foundation (grant no. 955/11), the Israel Cancer Research Foundation Research Career Development Award, The Dream Ideas Djerassi-Elias Institute of Oncology Research Fund, the Boaz and Varda Dotan Center Grant for Hemato-oncology Research and internal Tel-Aviv University funds.

EOSINOPHIL ACTIVATION AND MEDIATOR RELEASE

Peter F. Weller

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

Human eosinophils are distinguished among most other leukocytes by their capacity to package preformed cytokines and chemokines into intracellular granules available for very rapid, stimulus-induced secretion. Eosinophils secrete over 3 dozen cytokines through four distinct mechanisms. 1) In *piecemeal degranulation*, specific cytokines are selectively mobilized from intracellular granule stores into secretory vesicles that bud from intracellular granules. 2) In *classic exocytotic degranulation*, entire granules fuse with each other and/or the plasma membrane, causing wholesale release of granule contents. 3) In *cytolysis*, granules are extruded from eosinophils undergoing a cytolytic cell death, and deposited into the surrounding tissue. 4) In addition to secretion of pre-formed cytokines, eosinophils are stimulated to undergo *de novo* synthesis and secretion.

With a focus on granule-based secretion mechanisms involved in: a) piecemeal degranulation from within intact eosinophils and b) secretion from free extracellular eosinophil granules, both current understanding and issues yet to be delineated in regulated release of eosinophil-derived mediators will be considered.

Grant support: NIH R37 AI020241; R01AI022571; R01AI051645

EOSINOPHIL SURVIVAL: STATE OF THE ART

Francesca Levi-Schaffer[1]

[1] Pharmacology Unit, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel

Background: Understanding eosinophil survival and apoptosis is crucial to investigate the pathogenesis of eosinophilic inflammation and develop novel drugs to treat diseases associated with eosinophilia. Studies in this field have centered on the factors that keep eosinophils alive, with the pivotal discovery of the "eosinophils survival cytokines" (IL-3, IL-5 and GM-CSF). Several other factors have recently been identified, such as TNF-α, CysLTs, PGE2, leptin, IFNγ, neurotrophins, IL-25, IL-33 ,TSLP, IL- 27, spermine, low PH, etc.

In allergy, we investigate the soluble and physical mediated cross-talk between mast cells (MCs) and eosinophils that constitutes the "Allergic Effector Unit" (AEU). We have hypothesized that in the AEU both cells would have an enhanced phenotype including increased survival.

In the present work we aimed to define in the AEU novel mechanisms of enhanced eosinophils survival by specifically defining soluble mediators and the role of physical MC/Eos contact via CD48/2B4. Moreover we studied the influence of *S. aureus* and hypoxia (important allergic inflammatory microenvironmental factors) on eosinophil survival. Finally we checked in mice models of peritonitis and of atopic dermatitis (AD) (WT and KO mice for either 2B4 or CD48) the development of the disease as related to tissue eosinophilia.

Methods: Human cord blood and mouse bone marrow derived MCs and human peripheral blood and bone marrow derived eosinophils were cocultured (1-7 days) under different conditions. Eosinophils alone were also cultured in the presence of heat killed *S. aureus* or its exotoxins (SEB, PGN, PtA) or in 3% oxygen (hypoxic chamber). Eosinophil survival and activation were analysed (annexinV/PI, EPO, cytokine release, EM, signal transduction molecules, etc.). Peritonitis (compound 48/80 or SEB) and AD (OVA/SEB/tape stripping) were performed in WT or 2B4 or CD48 KO mice. Eosinophil numbers were evaluated in peritoneal lavages and skin together with other inflammatory parameters.

Results: In the AEU both soluble mediators (GM-CSF, IL-5, TNF-α, IL-3) and physical contact (mostly mediated by CD48/2B4) enhanced eosinophil survival in the absence of exogenous survival factor-s. Indeed on eosinophils in monocultures cross-linking of 2B4 or CD48 by mAbs also increased their viability. Moreover *S. aureus*/exotoxins binding to CD48 incremented significantly CD48 expression and eosinophil survival via MAPK phosphorylation. Similarly hypoxic conditions, in the absence or presence of GM-CSF, enhanced eosinophil survival by increasing Bcl-XL and HIF-1α expression. In 2B4 and CD48 KO mice peritonitis and AD inflammation were significantly reduced concomitantly with reduced eosinophil numbers.

Conclusions: We have shown novel mechanisms in allergic inflammation that contribute to eosinophil survival, i.e their cross-talk with MCs, their interaction with the bacteria *S. aureus* and hypoxic conditions.

Increased eosinophil survival is a main target for treatment of allergy and other diseases with eosinophilia. The best available drugs are still glucocorticosteroids that effectively induce eosinophil apoptosis. Novel drugs have not yet proven to be highly effective, perhaps also because they do not inhibit *in vivo* all the possible pro-survival factors present in the microenvironment. Therefore our

findings revealing novel mechanisms that can be targeted by specific therapy (such as CD48 with neutralizing mAbs) are important.

Grant support: This work was supported by MAARS EU 7th framework (grant no. HEALTH-F2-2011-261366), Israel Science Foundation (grant #213/05), and The Aimwell Charitable Trust (London, UK). FLS is a member of the David R. Bloom Center for Pharmacy and the Dr. Adolph and Klara Brettler Center for Research in Molecular Pharmacology and Therapeutics at The Hebrew University of Jerusalem.

EOSINOPHIL DEATH PATHWAYS

Hans-Uwe Simon

Institute of Pharmacology, University of Bern, CH-3010 Bern, Switzerland.

Eosinophils represent an essential cellular component of the innate immune system. They are constantly produced in the bone marrow, and similar numbers of eosinophils need to die within a defined time period in order to keep cellular homeostasis under physiologic conditions. Changing the rate of apoptosis rapidly changes cell numbers in such systems. In recent years, it became apparent that not all eosinophil death associated with inflammation is apoptosis. For instance, eosinophil cytolysis is often observed in eosinophilic tissues. It has been suggested that eosinophil cytolysis occurs without prior extensive degranulation and is the result of major activation mechanisms distinct from degranulation. Here, we describe molecular mechanisms of eosinophil activation resulting in cytolysis.

PATHOMECHANISMS 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. 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. A central role for innate epithelial responses regulated by esophageal specific caplain-14 is represented based on genetic and functional data. I will leave the message that EoE is a unique disease characterized by food hypersensitivity, strong hereditability influenced by early-life exposures and esophageal specific genetic risk variants, and allergic inflammation and that the disease is remitted by disrupting inflammatory and T-helper type 2 cytokine–mediated responses and through dietary elimination therapy.

CHARACTERIZATION OF PATHOGENIC TH2 CELLS IN EGID

Calman Prussin MD

Th2 cells express IL-4, IL-5 and IL-13 and play a fundamental role in allergy and asthma pathogenesis. We have previously characterized two human Th2 subpopulations, IL-5- and IL-5+ Th2 cells, the latter being a highly differentiated subset of Th2 cells. We identified hematopoietic prostaglandin D synthase (hPGDS) as the most highly up-regulated gene expressed by IL-5+ Th2 cells. We thus used a monoclonal antibody to hPGDS to identify and characterize IL-5+ Th2 cells as pathogenic effector Th2 (peTh2) cells. peTh2 cells expressed significantly greater IL-5 and IL-13 than did conventional Th2 (cTh2) cells. peTh2 cells were highly correlated with blood eosinophilia ($r = 0.78$-0.98) and were present in 30-40-fold greater numbers in EGID and AD vs. NA subjects. Relative to cTh2, peTh2 cells preferentially expressed receptors for TSLP, IL-25 and IL-33, and demonstrated greater responsiveness to these innate pro-Th2 cytokines. peTh2, but not cTh2 cells, produced PGD2. In EGID and AD, peTh2 cells respectively expressed gut and skin homing receptors. There were significantly greater numbers of peTh2 cells in gut tissue from EGID vs. NA controls. peTh2 cells are the primary functional pro-inflammatory human Th2 subpopulation underlying allergic eosinophilic inflammation. As such, peTh2 cells are a potential target in the treatment of allergic eosinophilic inflammation. The unambiguous phenotypic identification of human peTh2 cells provides a powerful tool to track these cells in future pathogenesis studies and clinical trials.

MECHANISMS OF TISSUE REMODELING IN EOE

Seema S. Aceves, MD, PhD

EoE is a chronic disease in children and adults associated with substantial eosinophil-associated tissue remodeling. Features of remodeling include epithelial basal zone hyperplasia, dilated intercellular spaces, and desquamation as well as subepithelial lamina propria fibrosis and angiogenesis. Remodeling is the underlying mechanism for EoE disease complications of strictures, food

impactions, and smooth muscle dysmotility. Eosinophils as well as mast cells seem to be integrally involved in the mechanisms of esophageal remodeling with the production of pro-fibrotic factors such as transforming growth factor beta-1 (TGFb1). TGFb1 in turn regulates the production of a number of fibrosis-associated products including periostin and MMP-14. In addition, TGFb1 can alter smooth muscle cell contraction. While inflammation and fibrosis appear to be coupled in children, dissociation can occur in some adults as well as in a subset of children. The clinical outcomes, treatment options, and mechanisms of long-standing or difficult to control fibrosis and remodeling warrant continued further investigation.

STUDIES ON THE ROLE OF EOSINOPHILS IN CHRONIC RHINOSINUSITIS (CRS)

Robert P. Schleimer and the Northwestern Sinus Center, Northwestern University Feinberg School of Medicine, Chicago Il, USA.

Chronic rhinosinusitis (CRS) is defined as chronic inflammation of sinonasal tissue of at least 12 weeks' duration and affects approximately 10 percent of the world's population. CRS is classified into subtypes based upon the presence of nasal polyps (NP) as CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP). Studies with anti-IL-5 suggest that eosinophils play a pathogenic role in the CRSwNP. In the US and Europe, NP tissue from CRSwNP patients is infiltrated by substantial numbers of eosinophils, while these cells are less numerous in Asian populations. While approximately half of CRS patients have asthma, approximately 10% of patients with CRS have both asthma and a hypersensitivity to medications that inhibit the cyclooxygenase 1 enzyme, a clinical triad known as Aspirin Exacerbated Respiratory Disease (AERD). We have identified over 170 AERD patients in the Northwestern health system and are studying pathogenic mechanisms in these patients. Some of the findings that will be discussed include: correlation of eosinophilia with type 2 cytokines and chemokines in CRS; correlation of eosinophilia with disease severity; production of CCL23 by eosinophils in CRS; evidence that eosinophils are responsible for olfactory dysfunction in CRS; relative lack of eosinophilia in CRSwNP patients of Asian descent suggesting genetic influences on eosinophilia; excess eosinophilia in patients with AERD; evidence for activation of eosinophil and basophil degranulation in AERD. The presentation will provide an overview of studies carried out in the Northwestern Sinus Center focusing on pathogenic mechanisms of CRS and relevant to the role of the eosinophil.

Grant support: supported by grants from the NIH and the Ernest S. Bazley Foundation

ALLERGIC FUNGAL AIRWAY DISEASE

Andrew Wardlaw and Catherine Pashley. Institute for Lung Health, Department of Infection Immunity and Inflammation, University of Leicester. UK

IgE sensitization to thermotolerant fungi such as *Aspergllus fumigatus* is common and associated with high levels of IgE (>1000KU/L) and often a marked peripheral blood eosinophilia. Indeed type 1 allergy to colonizing fungi is one of the commonest causes of referral to my hypereosinophilia clinic with a mean highest eosinophil count of ~$5X10^9$/L. A comprehensive screen for fungal allergy is therefore an important part of the work up of patients with an unexplained eosinophilia. Allergic fungal airways disease, defined as patients with airway symptoms and raised specific IgE to thermotolerant fungi, has a protean presentation including allergic bronchopulmonary aspergillosis (ABPA), difficult asthma, eosinophilic pneumonia, lobar collapse due to large airway obstruction, pneumonitis and late onset fixed airflow obstruction. A blood eosinophilia is a characteristic hallmark of all these presentations. The role of fungal allergy in airways disease has been dominated by the concept of allergic bronchopulmonary aspergillosis (ABPA). This is a complication of asthma and cystic fibrosis which was defined by a set of criteria, the main purpose of which was to distinguish between patients where allergy to *A. fumigatus* was likely to cause problems and those in whom it was thought to be benign. However we have demonstrated that asthmatics IgE sensitized to *A. fumigatus* who don't fulfill the criteria for ABPA are at increased risk of having fixed airflow obstruction and bronchiectasis. Moreover we have demonstrated that two of the criteria that define ABPA, a high specific IgG to *A fumigatus* and a high total IgE are not associated with markers of lung damage in allergic fungal airways disease. This suggests that the criteria for ABPA need to be revisited and that a term such as allergic fungal airway disease which captures all the patients in whom fungal allergy may cause lung damage are included. Many patients with asthma have features suggesting fungal allergy but no fungal sensitization can be detected. In addition fungal colonization can occur without allergy. We have therefore started to use high throughput sequencing to determine the full range of fungi colonizing the airway in asthma in order to describe the full spectrum of fungal species that may have a role in asthma. In preliminary data we have found the airway mycobiome to be dominated by *Aspergillus, Penicillium* and *Candida* species although we have identified over 200 fungal genera in airway samples many of which appear worthy of further examination.

ATOPIC DERMATITIS AND IMMUNODEFICIENCY

Joshua D. Milner, MD

Elevations in blood and tissue eosinophils can be found in a variety of monogenic diseases. Identifying syndromic patients or families with mendelian inheritance patterns associated with eosinophilia can substantially improve differential diagnoses for patients, and also provide molecular pathways of relevance which may help understand the pathogenesis of certain types of eosinophilia.

Atopy in general, and atopic dermatitis in particular, is the predominant symptom type in most cases of syndromic eosinophilia, although not the only type. Previously known syndromes associated with eosinophilia and atopic dermatitis have included autosomal dominant hyper-IgE syndrome, SPINK5 deficiency, DOCK8 deficiency, IPEX, filaggrin deficiency, Omenn's syndrome and Loewys-Dietz Syndrome and others. Recent advances in genomics have facilitated a marked increase in the number of identified genetic causes of syndromes associated with eosinophilia including, desmoglein deficiency and PGM3 deficiency. Study of patient clinical phenotypes, especially as they relate to end-organ atopic and eosinophilic manifestations, as well as molecular and cellular studies of these genetic disruptions can and have yielded new insights into the pathogenesis of atopic and eosinophilic pathways, although much more has yet to be understood.

Joshua D. Milner, MD Chief, Genetics and Pathogenesis of Allergy Section Laboratory of Allergic Diseases, NIAID, NIH

ILC2S AND EOSINOPHILIC INFLAMMATION

Doherty, Taylor A.[1]

Department of Medicine, UC San Diego. La Jolla, CA.

Background: Recently discovered group 2 innate lymphoid cells (ILC2s) potently produce Th2 cytokines. The purpose of our studies was to identify levels of ILC2s in human eosinophilic diseases (nasal polyposis and eosinophilic esophagitis) as well as determine ILC2 responses to lipid mediators.

Methods: We utilized flow cytometry to determine levels of lineage-negative CRTH2+ ILC2s in nasal polyps from adult patients with nasal polyposis and in esophageal biopsies from pediatric patients with eosinophilic esophagitis. We further assessed the role of CRTH2 in human ILC2 chemotaxis as well as cysteinyl leukotriene activation of mouse lung ILC2s. Finally, we determined whether airway challenges with leukotriene C4 (LTC4) given to mice could induce synergistically induce eosinophilic lung inflammation and ILC2 activation when administered with IL-33.

Results: We found strong correlations between tissue ILC2 levels and eosinophils in both nasal polyposis and active eosinophilic esophagitis. CRTH2 mediated human ILC2 chemotaxis to prostaglandin D2 and cysteinyl leukotrienes potently activated mouse ILC2s. Further, LTC4 potentiated IL-33-induced eosinophilic lung inflammation and lung ILC2 activation that was dependent on the CysLT1 receptor.

Conclusions: ILC2s are found in human eosinophilic diseases including nasal polyposis and eosinophilic esophagitis and correlate with levels of tissue eosinophilia. Additionally, lipid mediators including prostaglandin D2 and cysteinyl leukotrienes promote ILC2 responses.

Grant support: NIAID, AAAAI/ALA

GLYCOBIOLOGY AND EOSINOPHILIC LUNG INFLAMMATION: CONTROL PATHWAYS INVOLVING SIGLEC-F AND SIALYLATED GLYCANS CARRIED ON AIRWAY MUCINS.

Takumi Kiwamoto[1], Toshihiko Katoh[2], Christopher M. Evans[3], William J. Janssen[3], Zhou Zhu[1,4], Michael Tiemeyer[2] and Bruce S. Bochner[1,5]

[1]Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine; [2]Complex Carbohydrate Research Center, University of Georgia; [3]Division of Pulmonary Medicine, University of Colorado School of Medicine; [4]Section of Allergy and Immunology, Yale University School of Medicine; [5]Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine

Background: Siglec-F is a glycan binding protein expressed on mouse eosinophils and its engagement induces eosinophil apoptosis, suggesting a pathway for controlling eosinophil-associated diseases. Siglec-F recognizes specific a2,3-linked, sialylated, sulfated glycans in vitro, but the identities of endogenous glycoprotein ligands in vivo are unknown.

Methods: Lungs from normal and mucin-deficient mice, as well as cultured tracheal epithelial cells from mice (mTEC) were examined. Western blotting and immunocytochemistry was completed looking for Siglec-F-Fc binding glycoproteins. Liquid chromatography-tandem mass spectrometry analysis of Siglec-F-Fc binding glycoproteins was performed, and mouse eosinophil mucin binding and cell death assays were done using flow cytometry.

Results: We characterized glycoproteins isolated from mTEC and mouse lung tissue homogenates that bind to Siglec-F-Fc in a sialic acid dependent manner. Binding of Siglec-F-Fc to mTEC was sialidase-sensitive and was increased after treatment with IL-4 or IL-13. Sialidase-sensitive, PNGaseF-resistant binding of Siglec-F-Fc to glycoproteins of apparent MW ≈500 kDa and 200 kDa was detected by western blotting of mTEC lysates and culture supernatants, indicating the importance of sialylated O-linked glycoprotein glycans for Siglec-F binding. Binding was also resistant to solvolysis, suggesting that these endogenous ligands do not require sulfation for Siglec-F recognition. The expression of these glycoprotein ligands was increased during mouse allergic airways inflammation. Liquid chromatography-tandem mass spectrometry-based proteomics, cross-immunoprecipitation, and histochemical

analysis of lungs validated the identity of the glycoproteins as Muc5b, and also possibly Muc4. Muc5b null mice, but not Muc5ac null mice, had diminished Siglec-F airway ligands as determined by western blotting and immunohistochemistry. Purified mTEC mucins carried sialylated glycans, bound to eosinophils and induced their death in vitro. In vivo, BAL eosinophil numbers were increased, and apoptosis was decreased, in Muc5b conditional null mice after intratracheal administration of IL-13 compared to wild type mice.

Conclusion: These studies demonstrate that sialylated glycans displayed by the airway mucins Muc5b and Muc4 are endogenous ligands for Siglec-F and that Muc5b mediates Siglec-F dependent killing of mouse eosinophils in vitro and in vivo. Our data identify a previously unrecognized endogenous anti-inflammatory property of specific mucins by which their sialylated glycans can control lung eosinophilia through Siglec-F engagement.

STATE-OF-THE-ART: CURRENT TREATMENT OPTIONS AND UNMET NEEDS FOR EGID

Ikuo Hirano MD, Professor of Medicine, Northwestern University Feinberg School of Medicine

Eosinophilic gastrointestinal disorders (EGID) affect a variety of regions of the intestinal tract from the esophagus to the colon. Over the past two decades, eosinophilic esophagitis (EoE) has been increasingly recognized as one of the leading causes of dysphagia and foregut symptoms in children and adults. Prospective clinical trials have identified a number of medical, dietary and endoscopic therapies that are highly effective at remedying the symptoms, signs and histopathology of EoE. Current, first-line treatments for EoE include topical corticosteroids, elimination diets, and esophageal dilation. Biologic therapies targeting cytokines such as IL-5 and IL-13 are emerging as promising, systemic approaches. Therapy for non-EoE EGIDs remain inadequately studied. As the mechanisms underlying EGIDs are elucidated, novel therapies are rapidly evolving. Concurrently, the optimal endpoints for therapeutic response in EoE are being defined and refined. Patient reported outcome instruments are completing validation while the utility of novel physiologic and genetic biomarkers are investigated.

LINKS BETWEEN MICRORNAS AND THEIR MESSENGER RNAS POTENTIALLY REGULATE EOSINOPHIL DIFFERENTIATION

Ming Yang[1], Fiona Eyers[1], Ian G. Young[2], Helene F. Rosenberg[3] and Paul S. Foster[1]

[1]Priority Research Centre for Asthma and Respiratory Disease, Department of Microbiology and Immunology, School of Pharmacy and Biomedical Sciences, Faculty of Health and Hunter Medical Research Institute, University of Newcastle, Newcastle, Australia.; [2]Department of Molecular Biosciences, The John Curtin School of Medical Research, Australian National University, Canberra, Australia; [3]Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, United States of America

Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate complex transcriptional networks underpin immune responses. However, little is known about the specific miRNA networks that control differentiation of specific leukocyte subsets. In this study, we profiled miRNA expression during differentiation of eosinophils from bone marrow (BM) progenitors (bmEos), and correlated expression with potential mRNA targets involved in crucial regulatory functions. Profiling was performed on whole BM cultures to document the dynamic changes in miRNA expression in the BM microenvironment over the differentiation period. miRNA for network analysis were identified in BM cultures enriched in differentiating eosinophils, and chosen for their potential ability to target mRNA of factors that are known to play critical roles in eosinophil differentiation pathways or cell identify.

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

Results: We identified 68 miRNAs with expression patterns that were up- or down- regulated 5-fold or more during bmEos differentiation. By employing TargetScan and MeSH databases, we identified 348 transcripts involved in 30 canonical pathways as potentially regulated by these miRNAs. Furthermore, by applying miRanda and Ingenuity Pathways Analysis (IPA), we identified 13 specific miRNAs that are temporally associated with the expression of IL-5Ra and CCR3 and 14 miRNAs associated with the transcription factors GATA-1/2, PU.1 and C/EBPe. We have also identified 17 miRNAs that may regulate the expression of TLRs 4 and 13 during eosinophil differentiation, although we could identify no miRNAs targeting the prominent secretory effector, eosinophil major basic protein.

Conclusions: This is the first study to map changes in miRNA expression in whole BM cultures during the differentiation of eosinophils, and to predict functional links between miRNAs and their target mRNAs for the regulation of eosinophilopoiesis. Our findings provide an important resource that will promote the platform for further understanding of the role of these non-coding RNAs in the regulation of eosinophil differentiation and function.

THE ROLE OF EOSINOPHILS IN PROTECTION IMMUNOREGULATION AND PATHOLOGY DURING NEMATODE INFECTION.

E.T. Cadman, A.Y.N. Cheung, A. Davies-House, S. Bearder, J.J. Lee and R.A. Lawrence. Royal Veterinary College, University of London, UK.

During helminthic disease, the role of eosinophils can be both protective in immune responses and destructive in pathological responses. Using eosinophil-deficient mice (PHIL), we confirm the role of eosinophils in clearance of Brugia malayi microfilariae during primary, but not challenge infection in vivo. We also explored the role of eosinophils granule proteins, eosinophil peroxidase (EPO) and major basic protein-1 (MBP-1), during infection with microfilariae. Deletion of EPO or MBP-1 alone was insufficient to abrogate parasite clearance suggesting that either these molecules are redundant or eosinophils act indirectly in parasite clearance via augmentation of other protective responses. Absence of eosinophils increased mast cell recruitment, but not other cell types, into the broncho-alveolar lavage fluid during challenge infection. In addition absence of eosinophils or EPO alone, augmented parasite-induced IgE responses, demonstrating that eosinophils are involved in regulation of IgE. Whole body plethysmography indicated that nematode-induced changes in airway physiology were reduced in challenge infection in the absence of eosinophils and also during primary infection in the absence of EPO alone. However lack of eosinophils or MBP-1 actually increased goblet cell mucus production. We are currently exploring the role of regulatory cells in control of eosinophilia. Overall our results reveal that eosinophils actively participate in regulation of IgE and goblet cell mucus production via granule secretion during nematode-induced pathology and highlight their importance both as effector cells, as damage-inducing cells and as supervisory cells that shape both innate and adaptive immunity.

CUTTING EDGE: NOVEL EOSINOPHIL THERAPEUTIC TARGETS

Paneez Khoury

Eosinophil-associated disorders can present with blood and tissue eosinophilia with varying degrees of associated disease chronicity and organ dysfunction. Numerous studies ranging from clinical trials to animal models have provided evidence of the pathogenic role of eosinophils at sites of recruitment in these disorders. An increased knowledge of mechanism of eosinophil development, activation and trafficking has led to innovative strategies focused on interrupting downstream eosinophil effector functions. From investigational agents blocking eosinophil activation, survival, adhesion and recruitment, to agents targeting eosinophils and their progenitors for cell-death, new approaches to treat the range of eosinophil-associated disorders are on the horizon. Therapeutics in preclinical stages of development, clinical trials, and marketed agents will be discussed as well as their potential roles in treatment of eosinophil-associated disorders.

THE TREATMENT OF EOSINOPHIL-RELATED SKIN DISORDERS

Dagmar Simon

Department of Dermatology, Inselspital, Bern University Hospital, Bern, Switzerland

Eosinophil infiltration in the skin can be observed in allergic/immunologic, autoimmune, infectious and neoplastic disorders. Skin diseases with eosinophilia usually present with pruritus, while the cutaneous manifestations are variable. Principally, two main mechanisms leading to eosinophilia have been identified: a clonal eosinophil expansion (primary eosinophilia), and a reactive expansion due to extrinsic cytokine production by T cells or tumor cells (secondary eosinophilia). A careful clinical examination, histology, laboratory and imaging procedures in order to get the exact diagnosis and identify the cause of skin eosinophilia are a prerequisite for planning further treatment based on the pathophysiology. Hematopoietic stem cell disorders are treated according to hemato-/oncological protocols. Tyrosine kinase inhibitors are indicated in hypereosinophilic syndrome (HES) due to FIP1L1/PDGFRA, whereas an anti-IL-5 antibody therapy has been shown effective in non-FIP1L1/PDGFRA and clonal T cell HES. Eosinophilia in inflammatory skin diseases, lymphocytic forms of HES is treated with corticosteroids or other immunosuppressive drugs. Atopic dermatitis responds to the blocking of IL-4 an IL-13. An anti-CD52 antibody therapy is effective in Sezary syndrome, a CTCL. Novel substances targeting eosinophilia are currently under investigation.

THE ROLE OF TRIB1 IN EOSINOPHIL DEVELOPMENT

Ethan A. Mack[1], Sarah J. Stein[1], Kelly S. Rome[1], Warren S. Pear[1]

[1]Department of Pathology and Laboratory Medicine, Abramson Family Cancer Research Institute, The University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Background: The development of eosinophils proceeds with the concerted action of key transcription factors including C/EBP family members, GATA factors, and PU.1, yet how their expression is regulated is unclear. We and others identified tribbles homologue 1 (Trib1) as a critical regulator of C/EBPα protein turnover and others have shown that whole body Trib1-deficient mice lack eosinophils, but the developmental stage and mechanism of regulation remain unexplored.

Methods: We have generated mice conditionally deficient in Trib1 in the hematopoietic compartment using Vav-Cre (VCT1fl/fl). Using these mice, we employed both *in vivo* and *ex vivo* approaches to assess eosinophil development and regulation. Through flow cytometric analysis, we examined bone marrow, spleen, and peripheral compartments for eosinophils and other myeloid populations. In addition, we used *ex vivo* bone marrow cultures with IL-5 to generate eosinophils from these mice and quantitative PCR (qPCR) to profile these cells.

Results: We confirmed that VCT1fl/fl mice have a near complete absence of eosinophils in the bone marrow, spleen, and peripheral sites. This correlates with an expansion of neutrophils in these same sites. In addition, the efficiency of generating eosinophils *ex vivo* in culture with IL-5 is dramatically reduced compared to wild-type bone marrow, suggesting a cell-intrinsic defect. The few eosinophils generated from VCT1fl/fl bone marrow are grossly abnormal with reduced expression of eosinophil peroxidase mRNA and a more neutrophilic appearance on microscopic examination. Correlated with this is an increase in C/EBPα p42 protein in VCT1fl/fl whole bone marrow.

Conclusions: Our results suggest that Trib1 is a key regulator of eosinophil development as Trib1-deficient mice lack peripheral eosinophils. The reduced ability to generate eosinophils from bone marrow progenitors even under high IL-5 conditions suggests a differentiation block or survival defect. This is correlated with increased C/EBPα p42 in the bone marrow, suggesting that high levels of C/EBPα p42 arrest eosinophil development or inhibit survival.

Grant support: Alex's Lemonade Stand Foundation; The Waxman Foundation; NIH 2T32HL007439-36

THE TRANSCRIPTION FACTOR XBP1 IS SELECTIVELY REQUIRED FOR EOSINOPHIL DIFFERENTIATION

Sarah E. Bettigole[1,2], Raphael Lis[3,4], Stanley Adoro[2], Lisa A. Spencer[5], Peter F. Weller[5], Ann-Hwee Lee[6] and Laurie H. Glimcher[2*]

[1]Program in Immunology, Harvard Medical School, Boston, MA; [2]Department of Medicine, Weill Cornell Medical College, Cornell University, New York, NY; [3]Ansary Stem Cell Institute, Department of Genetic Medicine, and Howard Hughes Medical Institute, Weill Cornell Medical College, New York, New York 10065, USA; [4]Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, New York 10065, USA; [5]Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [6]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, Cornell University, New York, NY

Background: The canonical unfolded protein response (UPR) is a three-pronged signaling axis charged with correcting stresses within the endoplasmic reticulum. XBP1 is the most evolutionarily conserved branch of the UPR, and plays key roles in the development of highly secretory tissues such as plasma cells and Paneth cells. However, its function in granulocyte maturation remains unknown.

Methods: We employed conditional knockout mouse models, flow cytometry, *in vitro* culture systems, mixed bone marrow chimeras, RNA-sequencing transcriptome profiling, immunofluorescence and biochemistry to demonstrate that XBP1 and its upstream activator IRE1 are fundamentally required for murine eosinophil development.

Results: Targeted hematopoietic ablation of XBP1 or its upstream activator IRE1α using Vav1-Cre conditional knockout mice resulted in complete loss of mature eosinophils and dramatic decrease in Lin^-, $Sca1^-$, $CD34^+$, c-Kit^{lo}, IL-$5R\alpha^+$ eosinophil progenitors without altering neighboring hematopoietic lineages such as basophils and neutrophils. Myeloid and eosinophil progenitors selectively activated XBP1 without induction of parallel canonical ER stress signaling pathways. XBP1 was required after eosinophil lineage commitment in a cell-intrinsic manner to sustain cell viability. Unbiased bioinformatic analyses of transcriptional changes revealed that *Xbp1* deficiency reduced the adaptive protein folding capacity of the ER. Upon eosinophil commitment, this vulnerability led to massive defects in post-translational maturation of key granule proteins required for survival, and these unresolvable structural defects fed back to suppress critical lineage determining aspects of the transcriptional developmental program.

Conclusions: We present the first evidence that granulocyte subsets can be distinguished by their differential sensitivities to perturbations in XBP1-mediated secretory pathway functions. Furthermore, this work implicates the IRE1α/XBP1 signaling axis as a potential therapeutic target for eosinophil-mediated diseases.

EOSINOPHIL MEDIATORS CONTRIBUTE TO LUNG REMODELING AND DYSFUNCTION IN A MODEL OF SEVERE ASTHMA

Elizabeth A. Jacobsen[1], Sergei Ochkur[1], Wen Li[2], Dana Colbert [3], Katie R. Zellner[1], Cheryl Protheroe[1], James J. Lee [1] and Nancy A. Lee [3]

[1] Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259; [2] Zhejiang University School of Medicine, China; [3] Divisions of Hematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

Rationale: Chronic asthma is characterized by pulmonary remodeling and sustained lung dysfunction. Release of toxic secondary granule products by eosinophils has been suggested to lead to a majority of these lung remodeling events in patients. In particular, extensive release and accumulation of destructive secondary granule proteins eosinophil peroxidase (EPX) and major basic protein (MBP-1) are suggested to lead to epithelial damage and airways hyperresponsiveness. By using an eosinophil-dependent severe mouse model of chronic asthma crossed to mice deficient in granule proteins EPX and MBP-1 and in cytokines known to participate in lung remodeling/dysfunction these studies will highlight the individual role of these mediators in chronic severe asthma.

Methods: We developed a double transgenic strain of mice that express IL-5 from a CD3δ promoter (i.e., T cells) in addition to expressing human eotaxin 2 from the CC10 Clara cell promoter (I5/hE2). Expression of both transgenes leads to a chronic severe form of eosinophilic asthma that resembles many features that are found in asthmatic patients. These include significant histopathological remodeling (e.g., smooth muscle thickening, goblet metaplasia/epithelial mucus accumulation), increased airway hyperresponsiveness, and extensive eosinophil degranulation. These pathologies are eosinophil-dependent (i.e., they are absent in triple transgenic I5/hE2/PHIL) animals. We crossed these I5/hE2 mice to mice deficient in EPX, MBP-1, and IL-13 (I5/hE2/EPX$^{-/-}$, I5/hE2/MBP-1$^{-/-}$, I5/hE2/IL-13$^{-/-}$; respectively) to determine the relevant importance of these eosinophil mediators in lung remodeling, lung dysfunction, and degranulation.

Results: Deficiencies in MBP-1 or EPX did not affect the levels of degranulation, airways cellular infiltration, Th2 cytokine expression, or significant histopathological changes. However, airway hyperresponsiveness was mildly attenuated, although not reaching significance in these mice. In contrast, our data demonstrate that I5/hE2/IL-13$^{-/-}$ mice have reduced histopathologies and significantly improved lung function, even in the presence of extensive eosinophil secondary granule protein release.

Conclusion: Our findings highlight an unexpected result: Individually, the eosinophil secondary granule proteins EPX and MBP-1 have mild effects on both lung remodeling/function whereas the release of IL-13 is a significant inducer of the induced remodeling/lung dysfunction even in the presence of extensive eosinophil degranulation (i.e., widespread release of secondary granule proteins). Altogether these data indicate that eosinophils are not likely to have an exclusive and simple role as destructive mediators of disease, they instead appear to mediate complex immune/remodeling events possibly through IL-13 that lead to chronic airway pathologies.

This work is supported by Mayo Foundation for Medical Education and Research and grants from National Institutes of Health [NAL (HL058723), JJL (HL065228), and JJL (HL124165)] and American Heart Association [EAJ (11SDG7510043)].

PIN1 IS REQUIRED FOR EOSINOPHIL MIGRATION MEDIATED BY G PROTEIN-COUPLED RECEPTOR EBI2

Zhong-Jian Shen[1], Jie Hu[1], Stephane Esnault[2], Elizabeth A. Kelly[2], David Russell[3], and James Malter[1]

[1] Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas, United States; [2] Department of Medicine, Allergy, Pulmonary, and Critical Care Medicine Division, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, United States; [3] Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas, United States.

Eosinophil recruitment to sites of allergic inflammation is dependent on the local production of eosinophil-priming cytokines (e.g. GM-CSF and IL-5) and C-C chemokines (e.g. eotaxins, RANTES and MCP-3/4). Pin1, a prolyl *cis-trans* isomerase, plays a key role in the regulation of innate immunity and the expression and signaling of cytokines. Previously, we have shown that pharmacological blockade or genomic deletion of Pin1 prevents the development of experimental asthma in rodents. Here, we assessed the capacity of EBI2 to induce human eosinophil migration and, if so, whether Pin1 plays a role in this process. EBI2 (also known as GPR183) is a seven-transmembrane domain, orphan G protein-coupled receptor implicated in the regulation of B cell migration and T cell-dependent antibody responses. Expression of EBI2 was detected in peripheral blood eosinophils at both protein and mRNA levels irrespective of the presence of survival cytokine IL-5. Surprisingly, the levels of 7α,25-dihydroxycholesterol (7α,25-OHC) and 7α,27-dihydroxycholesterol (7α,27-OHC), the potent and selective agonists of EBI2 were significantly increased in the BALF 24 h post-segmental allergen (dust-mite) challenge of patients with mild asthma. In vitro, incubation of eosinophils with the 7α,25-OHC increased Pin1 isomerase activity without affecting IL-5 cell survival activity. 7α,25-OHC promoted transwell cell migration which was enhanced after cell priming with low dose of IL-5 (10 pM). However, this effect was almost completely blocked by dominant negative (DN) WW peptides of Pin1 but not by nonfunctional, mutant WW which does not block Pin1 function. Moreover, incuba-

tion of cells with pertussis toxin (PTX) or adenylate cyclase (AC) inhibitors also blocked ligand signaling, suggesting Gi-cAMP-dependent pathway contributes to EBI2 mediated eosinophil migration. In order to further understand the underlying mechanisms, several kinase inhibitors were incubated individually with IL-5-primed cells before adding EBI2 ligand. Among those (inhibitors for p38, Erk1/2, and JNK MAPKs, PI3K, PKC-a/b, and S6K), PD98059 (Erk inhibitor) completely blocked the ligand induced cell migration. Given these observation, we conclude that EBI2-oxysterol signaling direct eosinophil migration in vitro by activating Gi-cAMP-Erk1/2 pathway which is regulated by prolyl isomerase Pin1. Our findings may provide potential therapeutic targets to control blood eosinophil recruitment to the bronchial mucosa in asthmatics.

SPECIFIC SUBSETS OF KINASES MEDIATE SIGLEC-8 ENGAGEMENT-INDUCED ROS PRODUCTION AND APOPTOSIS IN EOSINOPHILS

Daniela Janevska, Jeremy A. O'Sullivan, Yun Cao, Bruce S. Bochner

Allergy-Immunology, Northwestern University, Chicago IL

Background: Siglecs (sialic acid-binding, immunoglobulin-like lectins) are type I transmembrane proteins expressed primarily on leukocytes. 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-activated eosinophils, Siglec-8 binding causes apoptosis with increased mitochondrial damage and ROS production, but exact signaling mechanisms are unknown.

Methods: Using a mAb (2C4) against Siglec-8 in combination with candidate signaling molecule inhibitors, we first examined Siglec-8-mediated apoptosis and ROS production after 24 hr IL-5 priming (30ng/mL) of human eosinophils by flow cytometry.

Results: We observed that 2C4-mediated eosinophil apoptosis was inhibited by PP1 (Src kinase inhibitor), ibrutinib (Btk inhibitor), LY294002 (PI3K inhibitor), and GF109203x (PKC inhibitor) at IC50's of 4.5 μM, 0.9 nM, 1.3 μM, and 2.5 μM respectively (n=3-5). Complete inhibition of ROS production occurred at similar IC90's. Western blot analysis following Siglec-8 cross-linking with 2C4 showed increased phosphorylation of Src, PI3K, and Bmx that was detectable within 15 min, with no detectable change in phosphorylation of Btk or PKC.

Conclusions: In summary, Src kinases, PI3K, and Bmx are involved in Siglec-8 mediated ROS production and apoptosis in IL-5-activated human eosinophils. While the sequence of events is yet to be determined, Siglec-8 mediated apoptosis in eosinophils involves the unexpected recruitment of molecules normally associated with cell survival.

Grant support: Funded by NIH grants R01AI72265 and P01 HL107151.

DISSECTING GLUCOCORTICOID (GC) PATHWAYS REGULATING EOSINOPHIL VIABILITY IN CYTOKINE-ACTIVATED EOSINOPHILS: ROLE OF PROTEIN PHOSHATASE 5 ON SUPPRESSION OF GC RECEPTOR AND ASK1 PHOSPHORYLATION

Konrad Pazdrak[1], Christof Straub, Susan Stafford[1], William Calhoun[2] and Alexander Kurosky[1]

[1] Department of Biochemistry and Molecular Biology and the UTMB National Heart, Lung, and Blood Institute Proteomics Center, University of Texas Medical Branch, Galveston, TX 77555 USA, [2] Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX 77555

Background: GCs are extremely effective in the treatment of eosinophili associated disorders, and eosinophils usually respond to GCs with apoptosis and suppression of effector functions in a manner that parallels the clinical response to GC therapy. In some patients, however, eosinophilia is resistant to GC treatment and the mechanism of GC resistance is presently unclear. Specific inflammatory conditions affecting GC signaling are believed to underlie insensitivity to therapeutic effects of GCs observed in certain patients with eosinophilic inflammation.

Objectives: Viability screening of eosinophils stimulated with proinflammatory cytokines in the presence of GC suggested a protective role for IL-2/IL-4 and IL-13/IFNγ against GC-induced apoptosis. Proteomic analysis of steroid-resistant eosinophils identified aberrant phosphorylation of GC receptor and Apoptosis Signaling Regulated Kinase 1 (Ask1) that interacted with Protein Phosphatase 5 (PP5). We hypothesize that resistance to GC-induced eosinophil apoptosis is mediated by GR-bound PP5 phosphatase and subsequent inactivation of the ASK1 signaling pathway.

Methods: Peripheral blood eosinophils were isolated as described [1] and activated with IL-2 and IL-4 for 24 h prior exposure to Dexamethasone (Dex) or Compound A (Cpd A) at the concentration of 10-100 nM for 1 h or 24 h for signal transduction and

apoptosis studies, respectively. The involvement of PP5 and ASK1 in GCR signaling was assessed upon inhibition of PP5 and ASK1 expression with specific siRNA. Eosinophil apoptosis was assessed upon staining with Annexin V and 7-ADD.

Results: Non-activated, quiescent eosinophils were found to be sensitive to GC treatment, entering apoptosis within hours of stimulation; this phenomenon was preceded by phosphorylation of GR (at Ser-203, Ser-211 and Ser-226) and Ask1 (at Thr-838). Inhibition of Ask1 expression inhibited DEX-induced eosinophil apoptosis. IL-2/IL-4- or IFNγ/IL-13-stimulated eosinophils remained viable for several days in the presence of Dex, however, Cpd A, a GR ligand with selective trans-repressive activity could still induced eosinophil apoptosis. Cells resistant to DEX-induced apoptosis showed activation of PP5 phosphatase, and diminished phosphorylation of GR and Ask1. Our studies revealed interactions of the GR with PP5, and Ask1, and we found that inhibition of PP5 restored functional phosphorylation of the ASK1 and reversed cytokine-induced resistance.

Conclusions: Our results establish a causative role for PP5 phosphatase in aberrant phosphorylation of the GC receptor and ASK1 kinase linking eosinophil's resistance to steroid-induced apoptosis with signaling events induced by proinflammatory cytokines.

Grant support: This work is funded by NIH/NHLBI N01-HV-00245 (to AK), and NIH/NCRR KL2RR029875 (to KP)

IL-1SS IN EOSINOPHIL-MEDIATED GASTROINTESTINAL HOMEOSTASIS AND IMMUNOGLOBULIN A PRODUCTION

YunJae Jung[1], Ting Wen[2], Melissa K. Mingler[2], Julie M. Caldwell[2], Yui-Hsi Wang[2], David D. Chaplin[3], Eun-Hee Lee[1], Myoung Ho Jang[4, 5], So-Youn Woo[6], Ju-Young Seoh[6], Masayuki Miyasaka[7], and Marc E. Rothenberg[2]

[1]Department of Microbiology, Graduate School of Medicine, Gachon University, Incheon 406-799, Republic of Korea; [2]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA; [3]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA; [4]Academy of Immunology and Microbiology, Institute for Basic Science, Pohang 790-784, Republic of Korea; [5]Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea; [6]Department of Microbiology, Graduate School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea; [7]WPI Immunology Frontier Research Center, Osaka University, Suita 565-0871, Japan

Background: Eosinophils are multifunctional leukocytes that reside in the gastrointestinal (GI) lamina propria (LP), where their basal function remains largely unexplored. The GI tract, exposed to potentially harmful commensals and pathogens, protects itself via production of IgA. In this study, we investigated the role of eosinophils in the production of immunoglobulin A (IgA) in the GI tract.

Methods: We examined IgA levels in mice with a selective deficiency of systemic eosinophils (ΔdblGATA or PHIL) or GI eosinophils (eotaxin-1/2 double–deficient or CC chemokine receptor 3–deficient). The effect of adoptively transferred small intestinal LP cells on IgA synthesis in ΔdblGATA mice was evaluated. The expression of mediators of IgA production and the composition of intestinal microflora were also analyzed in ΔdblGATA mice. We also used RNA sequencing to determine gene expression profiles associated with IgA class switching.

Results: IgA levels were decreased in serum and intestinal lavage of eosinophil-deficient mice with reduced mucus production and PP size, and alterations in commensal intestinal microbiota. The IgA decrease in ΔdblGATA mice was partially restored by adoptively transferred small intestinal LP cells of wild-type mice but not by LP cells of ΔdblGATA mice. Eosinophil-deficient mice showed reduced expression of mediators of secretory IgA production, including intestinal IL-1β, inducible nitric oxide synthase, lymphotoxin (LT) α, and LT-β, and reduced levels of ROR-γt$^+$ innate lymphoid cells (ILCs) while maintaining normal levels of APRIL, BAFF, and TGF-β. RNA sequencing of the small intestine of ΔdblGATA mice also revealed none of the down-regulated genes directly linked with IgA class switching (genes for APRIL, BAFF, TGF-ß, IL-6 and IL-10). GI eosinophils expressed a relatively high level of IL-1β, and IL-1β–deficient mice manifested the altered gene expression profiles observed in eosinophil-deficient ΔdblGATA mice and decreased levels of IgA$^+$ cells and ROR-γt$^+$ ILCs. Decrease of IL-1ß transcripts was also observed in the lung, fat, heart and muscle in ΔdblGATA mice implying various biologic roles of eosinophil-derived IL-1ß.

Conclusions: Eosinophils are crucial for homeostatic intestinal immune responses including IgA production through their expression of IL-1ß. Regulation of intestinal immune responses by eosinophils also involves eosinophil-dependent changes in commensal microbiota. This work suggests that eosinophils indirectly function by altering the intestinal microenvironment to be more favorable for IgA production rather than by secreting cytokines that directly facilitate IgA class switching.

PAIRED IMMUNOGLOBULIN-LIKE RECEPTOR B INHIBITS EOSINOPHIL ACCUMULATION AND ACTIVATION IN EOSINOPHILIC ESOPHAGITIS

Ben-Baruch-Morgenstern N1, *Mingler MK2, Stuck EM2, Besse JA2, Wen T2, Reichman H1 , Munitz A1,^, Rothenberg ME2,^*..

[1]-Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, The Tel-Aviv University, Ramat Aviv, 69978, Israel, [2-] Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH, 45229
^- These authors contributed equally to this work

Background: Eosinophilic esophagitis (EoE) is a Th2-mediated disease associated with eosinophilic infiltration, epithelial cell hyperplasia and tissue remodeling. IL-13 and eotaxins are critical in coordinating the influx of eosinophils into the esophagus. Nonetheless, pathways that are capable of counter regulating eosinophil accumulation and subsequent activation in the esophagus have been underexplored. Paired immunoglobulin-like receptor (PIR)-B is a cell surface immune inhibitory receptor that is expressed by eosinophils. We have previously demonstrated that PIR-B is a negative regulator of eotaxin-induced eosinophil recruitment and that the PIR-A-PIR-B axis constitutes a critical role in eosinophil development. While these data suggest key functions for PIR-B in eosinophil-associated pathologies, the role of PIR-B in EoE is yet to be defined.

Material and Methods: To define the cellular source accounting for esophageal PIR-B expression, polychromatic flow cytometric staining was conducted using single cell suspensions obtained from the esophagus of *CC10-Il13Tg* mice. In order to define the role of PIR-B in IL-13-induced esophageal pathology, *Pirb-/-* mice were mated with *CC10-Il13Tg* mice to generate *CC10-Il13Tg/Pirb-/-* and *CC10-Il13Tg/Pirb+/+* mice. Thereafter, the mice were fed with doxycycline (dox) for 2 weeks and eosinophilic infiltration into the esophagus was determined (flow cytometry and IHC). Esophageal pathology including collagen deposition, myofibroblast formation, epithelial cell hyperplasia and angiogenesis were determined (IHC and qPCR). Transcriptome signature of purified primary esophageal eosinophils from *CC10-Il13Tg/Pirb-/-* and *CC10-Il13Tg/Pirb+/+* was determined by microarray analysis (Mouse Affymetrix 2.0 ST GeneChip®).

Results: The expression of PIR-B was increased in the esophagus following inducible overexpression of IL-13. In these settings, PIR-B was highly expressed by esophageal eosinophils, which constituted the majority of $CD45^+$ cells in the esophagus. Doxycycline-treated $CC10\text{-}Il13^{Tg}/Pirb^{-/-}$ mice displayed markedly increased esophageal eosinophilia and EoE pathology including increased epithelial cell hyperplasia, fibrosis, collagen deposition, myofibroblast formation and angiogenesis compared with $CC10\text{-}Il13^{Tg}/Pirb^{+/+}$ mice. ERK phosphorylation assays revealed that PIR-B was an intrinsic negative regulator of the esophageal microenvironment in response to IL-13 as in response to similar environmental triggers, *Pirb-/-* eosinophils display increased phosphorylation of ERK. Finally, global transcriptome analysis of primary sorted $Pirb^{+/+}$ and $Pirb^{-/-}$ esophageal eosinophils revealed increased expression of multiple transcripts associated with induction of tissue remodeling and cellular activation in $Pirb^{-/-}$ eosinophils including cell surface molecules (e.g. IL5Ra and BmpR2), cell adhesion and migration molecules (e.g. ezrin and actinα1), various enzymatic pathways (e.g. MMP9 and Capn2), secreted factors (e.g. IL-6, TGFβi) and intracellular signaling pathways (e.g. STAT6, NFKB signaling and Jun).

Conclusion: These data demonstrate that PIR-B is a negative regulator of IL-13-induced esophageal pathology likely by regulating eosinophil effector functions. Specifically, our data highlights PIR-B as a key molecular checkpoint in IL-13-induced eosinophil accumulation and subsequent activation, which may serve as a novel target for future therapy in EoE.

Grant support: US-Israel Bi-National Science Foundation

RESIDENT INTESTINAL EOSINOPHILS ACQUIRE LUMINAL ANTIGEN THROUGH LOW AFFINITY FC GAMMA RECEPTORS

Kalmia S. Buels, Raiann Rahman, and Lisa A. Spencer

Division of Allergy and Inflammation, Beth Israel Deaconess Medical Center, Boston, MA

Background: At baseline in healthy individuals, the greatest density of eosinophils is localized to the gastrointestinal tract, and numbers of intestinal eosinophils are further increased in several inflammatory diseases, including food allergies. In allergic diseases and helminth infections eosinophils have been shown to contribute to immunity through both antigen-independent and antigen-dependent mechanisms. Few studies have directly investigated resident tissue populations of eosinophils, therefore the precise function(s) of resident eosinophils within the intestinal niche in health and disease remain enigmatic.

Objective: In this study we queried whether intestinal tissue eosinophils interact directly with luminal-derived antigens *in vivo*.

Methods: *In vivo* uptake of luminal antigen by intestinal eosinophils and dendritic cells in live mice was measured using a surgical model wherein naïve or antigen-sensitized mice were anesthetized, and 5 cm segments of the terminal ileum sutured to form intestinal "loops". Forty-five minutes after injection of fluorescently labeled antigen into the lumen of the loops, mice were sacrificed, loops excised, Peyer's patches removed, and intestinal tissue disaggregated to form a single cell suspension. Cellular uptake of the fluorescently labeled antigen was determined by flow cytometry. For *in vitro* studies, total intestinal leukocytes, or resident

tissue eosinophils alone were isolated from the small intestines of wild-type mice and exposed *ex vivo* to antigen in the presence or absence of immune or naïve serum or purified IgG, and with or without Fc receptor inhibitors. Uptake of fluorescently labeled antigen was assessed by flow cytometry.

Results: Intestinal eosinophils from sensitized, but not naïve, mice acquired luminal- derived antigen. Antigen acquisition by tissue eosinophils was restricted to the sensitizing antigen. Intestinal eosinophils from antibody-deficient JH mice failed to acquire antigen, but eosinophil antigen acquisition remained intact in IgE-/- mice. Passive transfer of immune serum (but not naïve serum), or antigen-specific IgG enabled antigen acquisition by eosinophils in otherwise naïve mice. *In vitro* studies confirmed *in vivo* results; inclusion of immune serum or purified immune IgG enhanced antigen acquisition by intestinal eosinophils *ex vivo*. Heat inactivation of serum had no effect on antigen acquisition, suggesting complement does not play a role. In contrast, enhanced antigen acquisition was completely blocked by neutralizing antibodies against the low affinity IgG receptor, FcγRIII.

Conclusions: Antigen-specific IgG enables resident tissue eosinophils to acquire antigens from the small intestine lumen of mice *in vivo* through eosinophil-expressed FcγRIII. These data provide a direct mechanistic link between humoral adaptive immunity and resident intestinal eosinophils, and suggest tissue eosinophils may engage in antigen- dependent responses *in situ*.

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

ONCOSTATIN M IS ELEVATED IN PATIENTS WITH EOSINOPHILIC MUCOSAL DISEASE AND PROMOTES EPITHELIAL BARRIER DYSFUNCTION

Kathryn L. Pothoven[1] MSc, Kathryn E. Hulse[1] PhD, Paul J. Bryce[1] PhD, Bruce K. Tan[2] MD MS, Robert P. Schleimer[1,2] PhD

[1] Division of Allergy-Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Ill, [2] Department of Otolaryngology, Northwestern University Feinberg School of Medicine, Chicago, Ill

Background: Epithelial barrier dysfunction is thought to play a role in many eosinophilic mucosal diseases including asthma, chronic rhinosinusitis (CRS), and eosinophilic esophagitis (EoE).

Methods: Oncostatin M (OSM) expression was measured in tissue extracts, nasal secretions, and bronchoalveolar lavage (BAL). The effect of OSM stimulation on the barrier function of fully differentiated normal human bronchial epithelial cells and nasal epithelial cells cultured at air liquid interface (ALI) were assessed using transepithelial electrical resistance (TEER) and FITC dextran flux. Dual color immunofluorescence was used to evaluate integrity of tight junction structures in cultured epithelial cells.

Results: Analysis of CRS samples showed that OSM mRNA and protein were highly increased in nasal polyps compared to control uncinate tissue ($p<0.01$). OSM was also elevated in BAL of allergic asthmatics following segmental allergen challenge and in esophageal biopsies from EoE patients. OSM stimulation of ALI cultures resulted in reduced barrier function measured by decreased TEER and increased FITC dextran flux ($p<0.01$). Alterations in barrier function by OSM were reversible, and the viability of epithelial cells was unaffected. OSM levels in lysates of nasal polyps and UT positively correlated with α2-macroglobulin, a marker of epithelial leak, in localized nasal secretions ($r=0.6156$, $p<0.01$). OSM protein in the BAL of allergic asthmatics also correlated with human serum albumin another marker of epithelial leak ($r=.8062$, $p<.001$).

Conclusions: These results suggest that OSM may play a role in the epithelial barrier dysfunction that is seen in eosinophilic mucosal disease, and inhibition of OSM and/or its downstream effects may be beneficial in the treatment of these diseases through the potential restoration of mucosal epithelial barrier function.

EOSINOPHILS REGULATE AIRWAY NERVE SUBSTANCE P EXPRESSION

Katie M. Lebold[1,2], Matthew G. Drake[1], Emily D. Blum[1], Gregory D. Scott[1], Allison D. Fryer[1], Nancy A. Lee[3], James J. Lee[4], and David B. Jacoby[1]

[1]Pulmonary and Critical Care Department, Oregon Health and Science University, Portland, OR, [2]Biomedical Engineering Department, Oregon Health and Science University, Portland, OR, [3]Division of Hematology and Oncology; [4]Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic in Arizona, Scottsdale, AZ

Background: Airway nerves are dysfunctional in asthma and thus we sought to define eosinophil-mediated effects on nerve phenotype and neuropeptide expression.

Methods: Eosinphil-deficient (PHIL) and wild-type (WT, C57BL/6) mice were sensitized intranasally with 50μg of house dust mite (HDM) or saline on days 0 and 1 and challenged with 25μg HDM or saline on days 14-17. Animals were euthanized 24 hours after last HDM or saline exposure. Untreated interleukin-5 transgenic (specifically in airway epithelium; IL-5tg) mice were used as comparator model of airway eosinophilic inflammation. Whole mount tracheas were immunofluorescently labeled using antibodies to the pan-neuronal marker PGP9.5 and to the tachykinin substance P. Tracheas were optically cleared by dehydration in ethanol

followed by rehydration with benzyl alcohol/benzyl benzoate. Images were obtained with a Zeiss LSM780 laser-scanning confocal microscope. Three-dimensional computer models of airway nerves were generated and used to calculate substance P pixel intensity and nerve volume. Whole mount lungs from untreated WT, IL-5tg, and eosinophil-deficient IL-5 transgenic (IL- 5tg/PHIL) mice were prepared, imaged, and analyzed as above.

Results: HDM exposure resulted in eosinophilic airway inflammation in WT mice ($p<0.0001$) and neutrophilic airway inflammation in PHIL mice ($p<0.008$) compared with saline controls. HDM exposure increased tracheal epithelial nerve substance P expression ($p<0.0007$) in WT mice, but not in PHIL mice. HDM exposure did not change the percentage of epithelial nerves expressing substance P. IL-5tg mice have increased lung subepithelial nerve substance P expression ($p=0.002$) and percentage of substance P positive nerves ($p=0.0008$) compared with untreated IL-5tg/PHIL and WT mice. IL- 5tg/PHIL mice have reduced percentage of nerves expressing substance P compared with WT; however, this did not reach statistical significance.

Conclusions: Eosinophils increase the amount of substance P expressed within a nerve as well as the number of nerves producing substance P. In contrast, neutrophilic inflammation did not affect substance P expression. Eosinophil-dependent changes in airway nerve phenotype may contribute to nerve dysfunction in asthmatic patients.

Grant support: This project was supported by NIH grants HL121254, HL058723, HL124165, HL113023, AR061567

EOSINOPHILS STIMULATE NEUROPLASTICITY IN SENSORY NERVES

Quinn R. Roth-Carter[1], Allison D. Fryer[1], Nancy A. Lee[2], James J. Lee[2], and David B. Jacoby[1]

[1]Division of Pulmonary and Critical Care Medicine, Oregon Health & Sciences University, Portland, OR 97239, and [2]Mayo Clinic, Scottsdale AZ.

Background: Chronic itch is one of the main symptoms of atopic dermatitis, and constant scratching by patients disrupts skin barrier function, leading to increased inflammation, and worsening of the disease. Levels of eosinophil granule proteins are elevated in the skin of patients with atopic dermatitis, and eosinophils are required for itch in allergic contact dermatitis models. Eosinophils interact with sensory nerves in the skin in patients with atopic dermatitis, which is significant because sensory nerves mediate itch. The purpose of this study was to test whether eosinophils directly mediate changes in sensory nerve function observed in atopic dermatitis.

Methods: Sensory nerves were isolated from mouse dorsal root ganglia, dispersed into single cells, and grown in cell culture for 1 day. Mouse eosinophils were then isolated from the blood of NJ 1638 mice, which have IL-5 constitutively expressed under a T cell specific promoter leading to large numbers of eosinophils in the blood. Sensory nerve cultures were treated with either (1) resting eosinophils (freshly isolated), (2) eosinophils plus IL-4, IL-33 and GM-CSF to activate the eosinophils, or (3) media conditioned by activated eosinophils. After 24 hours eosinophils were washed away, and RNA was isolated from the sensory nerves. Gene expression for TRPV1, Thymic Stromal Lymphopoietin Receptor (TSLPR) and Substance P (TAC1) were analyzed using real time RT-PCR.

Results: Co-culture with resting eosinophils increased gene expression of TSLPR, but not TRPV1 or TAC1, in sensory nerves compared to untreated sensory nerves. Treating nerves with media conditioned by activated eosinophils led to an increase in TSLPR and TRPV1, but not TAC1, expression in sensory nerves when compared to cytokines alone. This demonstrates that activation of eosinophils changes the effect on sensory nerves, as resting eosinophils do not stimulate expression of TRPV1 in sensory nerves. Additionally, this data also shows that eosinophils increase expression of these receptors through release of a soluble factor, and that cell-cell contact is not required.

Conclusions: Taken together these data demonstrate that eosinophils directly stimulate expression of TRPV1 and TSLPR in sensory nerves through the release of a soluble factor. These findings identify eosinophil nerve interactions as a therapeutic target for the treatment of atopic dermatitis.

Grant support: This research was funded by the following grants; HL113023 (DBJ), AR061567 (DBJ+JJL), HL124165 (DBJ+JJL), HL058723 (NAL), Health Effects Institute (ADF), ES017593 (ADF) ARCS Scholarship, Pulmonary and Critical Care Medicine T32 Award

CD3-CD4+ T CELL-ASSOCIATED URTICARIA AND ANGIOEDEMA WITHOUT EOSINOPHILIA : A NEW BENIGN LYMPHOPROLIFERATIVE DISORDER

Caroline Carpentier[1], Soizic Garaud[2], André Efira[3], Liliane Schandené[4], Barbara Dessars[5], Karen Willard-Gallo[2], Elie Cogan[1], and Florence Roufosse[1,6]

[1]Department of Internal Medicine, Hôpital Erasme, Université Libre de Bruxelles (ULB); [2]Laboratory of Molecular Immunology, Institut Bordet, ULB; [3]Department of Hematology, Centre hospitalo-universitaire Brugmann, Brussels; [4]Laboratory of Immunology, Hôpital Erasme, ULB; [5]Department of Medical Genetics, Hôpital Erasme, ULB; [6]Institute for Medical Immunology, ULB - Brussels, Belgium.

Background. Clonal CD3-CD4+ T-cells have been described in peripheral T cell lymphoma, and lymphocytic variant hypereosinophilic syndrome (L-HES), a benign lymphoproliferative disorder that may present as episodic angioedema with eosinophilia. The hypereosinophilia (HE) typically observed in CD3-CD4+ T-cell-driven disease is due to marked IL-5 over-production by these cells, and is thought to contribute to the frequently observed cutaneous manifestations. We detected CD3-CD4+ T-cells in peripheral blood from 4 patients with recurrent urticaria and angioedema *without* HE (UAw/oEos). In this study, we further characterize this previously undescribed CD3-CD4+ T-cell mediated disorder.

Methods. Peripheral blood mononuclear cells were isolated from patients with UAw/oEos (n=4), L-HES (n=4), and 4 healthy subjects. CD3-CD4+ cells were purified by immuno-magnetic separation or cell-sorting; clonality was assessed by PCR for TCR gene rearrangements. T-cell surface phenotype and intracellular cytokine expression were studied by flow cytometry. Serum mediators were quantified with ELISA kits, or with a bead-based immuno-assay using flow cytometry. The latter was also used to determine cytokine concentrations in supernatants of in vitro-stimulated PBMC.

Results. Peripheral blood T-cell phenotyping revealed CD3-CD4+ cells in 4 patients with recurrent UAw/oEos, representing 0.51-13% of lymphocytes (absolute counts 13, 14, 31 and 83/mm^3). Purified circulating CD3-CD4+ T cells were clonal in all 4 cases. Biopsies of affected skin from 2 patients showed sparse lymphocytic infiltrates, no eosinophils, and PCR detected the same clone as that found in blood. The CD3-CD4+ cells were consistently CD45RO+CD2+CD5+CD95+. They stained positively for intracellular IL-2 (16-37%), but not for IFNγ or IL-6, similar to L-HES patients. Expression of IL-4 and IL-5 was barely detectable, as in healthy CD4 T-cells, and contrasting with L-HES. Intracellular IL-13 was slightly increased relative to healthy CD4 cells (median 5.5 versus 2,6%), but much lower than in L-HES patients (44,5%). Serum sCD25 levels were similar to controls (median 482 versus 564 pg/ml), and lower than in L-HES (median 2704, $p<0.05$). Serum IgM and tryptase were normal, whereas IgE was elevated in all 4 patients (227-36630 U/ml), and TARC levels tended to be higher than in controls (median 1326 versus 176 pg/ml, ns). IL-2, IL-4, IL-5, IL-9, IL-13 were undetectable in serum; only IL-6 was found at low levels in 2/4 UAw/oEos patients. Supernatants of in vitro-stimulated PBMC contained similar amounts of IL-2, IL-4, IL-5, IL-6, IL-9, and IL-13 to PBMC from healthy controls; in vitro stimulated CD3-CD4+ cells from one patient released IL-2 but not IFNγ, and no evidence for increased production of Th2 cytokines was found.

Conclusions. We report a new clinical condition associated with clonal CD3-CD4+ T cells, characterized by recurrent episodes of urticaria and angioedema in the absence of HE, in accordance with their inability to produce IL-5 in vitro. Although the aberrant cells are found in affected skin, the mechanisms whereby they contribute to symptoms remain elusive, as do their interactions with other cell types such as mast cells. Investigations are in progress to further explore CD3-CD4+ T-cell biology, and identify mediators that could represent future therapeutic targets. Our findings suggest that T cell phenotyping should systematically be performed in patients presenting with chronic urticaria and angioedema.

Grant Support. The present study was funded by the Belgian National Fund for Scientific Research (FNRS, grant 5/4/150/5) and by the NIF Trust.

IMMUNE REGULATORY SIGLEC LIGANDS AND THEIR UPREGULATION IN INFLAMED HUMAN AIRWAYS

Steve M. Fernandes[1], Yi Jia1[-2], Huifeng Yu[1], Yadong Wei[1], Anabel Gonzalez-Gil[1], Mary Motari[1,] Katarina Vajn[1,] Robert P. Schleimer [3,] Ronald L. Schnaar[1]

[1]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, [2]Department of Pharmacology, Third Military University, ChongQing, China, [3]Division of Allergy and Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

Background: Sialic acid-binding immunoglobulin-type lectins (Siglecs) are immune regulatory molecules found on the surfaces of inflammatory cells including Siglec-8 on eosinophils and mast cells, and Siglec-9 on neutrophils. When engaged by their sialoglycan ligands on target tissues, Siglec-8 and Siglec-9 induce apoptosis of the inflammatory cells on which they are expressed, down-regulating ongoing immune responses. Naturally occurring sialoglycan ligands on human tissues, including airways, have not been characterized.

Methods: Expressed tagged versions of human Siglec-8 and Siglec-9 (human Fc chimeras) were used to probe for Siglec binding partners (ligands) on chemically defined arrays of synthetic and natural sialoglycans and on human airway tissues. Sinonasal tissues

(inferior turbinate and uncinate) were obtained during routine endoscopic sinus surgery from non-inflamed subjects and from patients with chronic rhinosinusitis (CRS) with and without polyps. Lung parenchyma, trachea and bronchi were obtained postmortem from organ donors. The distribution of endogenous counter receptors for Siglec-8 and Siglec-9 in human airways was explored by overlay histochemistry using the expressed Fc chimeras.

Results: Glycan array studies revealed highly specific Siglec-8-Fc binding to a particular sulfated sialylated glycan structure, 6'-sulfosialyl-LacNAc (NeuAcα2-3(6-SO3) Galβ1-4GlcNAc), whereas Siglec-9 had a different and broader sialoglycan binding specificity. Using upper airway tissues, Siglec-8-Fc bound very selectively to a subpopulation of submucosal gland cells with the characteristics of submucosal serous cells. Siglec-8-Fc binding to lung tissues confirmed selective binding to submucosal serous cells, as well as to cartilage. Siglec-9-Fc bound more broadly to submucosal serous cells, airway epithelium, and connective tissue in both upper airway and lung. Binding of Siglecs to human tissues was absent after sialidase treatment. Comparing Siglec-8-Fc binding to normal versus inflamed (CRS) tissues revealed a marked (>2.5-fold) and significant ($P < 0.03$) increase in CRS while binding remained in submucosal glands and ducts. Siglec-9-Fc binding was also significantly increased (~70%) in submucosal glands, connective tissue and epithelium.

Conclusions: We found that sialoglycan ligands for Siglec-8 and Siglec-9 were present in submucosal glands and upregulated in chronically inflamed upper airways. Major Siglec-8 ligands appear to be synthesized in serous cells of the submucosal gland and secreted in response to inflammation. Siglec-9 ligands share this distribution, but are also found more broadly in airway tissues. The enhanced biosynthesis of inhibitory siglec ligands may be a tissue-level response that contributes to the control of airway inflammation. These distributions inform ongoing experiments to understand the expression of lung sialoglycans in the control of inflammation. Ongoing characterization of these endogenous siglec glycan ligands and their expression on normal and inflamed human tissues may provide novel opportunities to enhance or mimic their anti-inflammatory actions.

Grant support: Supported by the Lung Inflammatory Disease Program of Excellence (P01 HL107151), by R37 HL068546 and P01 AI106683 from the NIH, and by the Ernest S. Bazley Foundation.

INCREASED NUMBERS OF LL-5+IL-13+ GROUP 2 INNATE LYMPHOID CELLS IN SPUTUM OF STEROID DEPENDENT SEVERE ASTHMATICS WITH PERSISITENT AIRWAY EOSINOPHILIA

SG. Smith[1], R. Chen[1], M. Kjarsgaard[1], C. Huang[1], J-P. Oliveria[1], PM. O'Byrne[1], GM. Gauvreau[1], L-P. Boulet[2], C. Lemiere[3], J. Martin[4], P. Nair[1] and R. Sehmi[1]

[1]Dept Medicine, McMaster University, Canada, [2]Inst. Univ de cardiologie et de pneumologie de Québec, Canada, [3]Div Pneumologie, U Montreal, Canada, [4]Meakins-Christie Laboratories, McGill U Health Center, Canada

Background: In severe eosinophilic asthma, local maturation of lineage-committed progenitor cells maybe the predominant pathophysiological process that drives persistent airway eosinophilia. Increased numbers of eosinophil-lineage committed progenitor cells are present in the airways of severe asthmatics compared to mild asthmatics. We propose that local IL-5 driven extramedullary hematopoiesis stimulates persistent airway eosinophilia in steroid-dependent severe asthma. A novel cell type, group 2 innate lymphoid cells (ILC2) are a major source of type 2 cytokines (IL-5 and IL-13) and have been shown drive eosinophilic inflammatory responses in the absence of CD4+ lymphocytes in mouse models asthma. Increased absolute numbers of ILC2s have been found in the blood and airways of asthmatics subjects. Little however is known about the role of these cells in human eosinophilic asthma. This study enumerated absolute and intracellular cytokine positive ILC2s compared to other type 2 cells to better understand the role that these cells play in driving chronic airway eosinophilia in severe asthmatics despite regular high-dose oral corticosteroid therapy.

Methods: In a cross-sectional study, we enumerated blood and sputum-were collected from severe asthmatics (n=25), mild atopic asthmatics (n=19) and non-atopic healthy controls (n=5). All severe asthmatics had pre-bronchodilator FEV1<80% predicted, >12% reversibility of FEV1, PC20 <8mg/ml, at least 6 months history of treatment with oral steroids (5-35mg daily prednisone or equivalent), inhaled corticosteroids (>880mcg/day) and blood eosinophilia ≥150/μl currently or <300/μl within the past 12 months. Mild asthmatics were skin prick test positive, FEV1≥70% predicted, >12% reversibility of FEV1, PC20≤16 mg/ml, and were steroid-naïve with infrequent use of inhaled ß2-agonists. Absolute numbers of ILC2s (lin-CD45+127+ST2+) and cells expressing intracellular type 2 cytokines, IL-5 and IL-13 were assessed in blood and sputum samples. These levels were compared to type 2 immune cells including CD3+CD4+ lymphocytes and eosinophil-lineage committed progenitors (EoP; CD34+45+125+). Freshly isolated cells were immunostained for flow cytometric analyses without further ex-vivo stimulation.

Results: Significantly greater numbers of total and type 2 cytokine producing ILC2s were detected in blood and sputum of severe asthmatics compared to mild asthmatics and normal controls. In contrast, sputum levels of CD4+ and EoPs expressing intracellular IL-5+ or IL-13+ were not significantly different between the asthmatic groups but were greater than normal controls. In severe asthmatics although sputum CD4+ cells were the most abundant cells, ILC2s were proportionally the predominant source of IL-5

and IL-13. In addition, significantly greater levels of IL-5+IL-13+ ILC2s were detected within the airways of severe asthmatics whose sputum eosinophilia was greater than 3%, despite normal blood eosinophil levels (< 300/ µl).

Conclusions: The findings of this study suggest that increased local production of type 2 cytokines, namely IL-5 and IL-13, by luminal ILC2s may represent a steroid insensitive population that can drive persistent airway eosinophilia through local extramedullary eosinophilopoietic processes in severe prednisone-dependent asthmatics.

Grant support: Dr. SG Smith holds a post-doctoral fellowship award from the Father Sean O'Sullivan Research Centre, Hamilton. This research was supported by a Grant-In-Aid from the Canadian Lung Association awarded to Dr. R Sehmi.

CLAUDIN-1 DYSREGULATION IN EOSINOPHILIC ESOPHAGITIS: A ROLE FOR HIF-1A IN ESOPHAGEAL EPITHELIAL BARRIER DYSFUNCTION.

Joanne C. Masterson[1,2], Kathryn Biette[1,2], Bejan Saeedi[2], Rachel Harris[1,2], Shahan D. Fernando[1,2], Lindsay Hosford[1,2], Caleb J. Kelly[2], Eric L. Campbell[2], Stefan Ehrentraut[2], Eóin N. McNamee[2], Louise E. Glover[2], Sean P. Colgan[2], Glenn T. Furuta[1,2].

[1]Gastrointestinal Eosinophilic Diseases Program, Digestive Health Institute, Children's Hospital Colorado, [2]Mucosal Inflammation Program, University of Colorado, CO, USA.

Background: The emerging allergic condition EoE is increasing in prevalence. While the precise cause and pathophysiology of EoE is unknown, altered barrier function is implicated. HIF signaling has previously been connected to barrier function. We hypothesized that chronic inflammatory hypoxia, such as that elicited in chronic EoE, may result in dysregulated HIF signaling, thus altering epithelial barrier.

Methods: Human esophageal epithelial cells (EPC2-hTERT) were exposed to chronic hypoxia (1% O2 >36hrs) to assess the molecular and functional effects on HIF signaling and epithelial barrier. Eosinophils were isolated from healthy donors, stimulated with IL-5 and GM-CSF (100pg/ml) and subjected to oxygen-consumption assays. Expression of HIF-1α mRNA and protein, and tight junction molecules were assessed in esophageal pinch biopsies from active EoE subjects and our mouse model of EoE (L2-IL-5^{OXA}). Control EPC2-cells, EPC2-cells over-expressing a non-signalling dominant-negative-HIF-1α, and HIF-1α-knock-down EPC2-cells were utilized to assess barrier function by transepithelial electrical resistance (TEER) and FITC-Flux assays in a 3-dimensional air-liquid interface (3D-ALI) model that induces stratification and differentiation of epithelial cells *in vitro*. Chromatin-immunoprecipitation (ChIP) was performed in addition to promoter-mutagenesis luciferase assays to study HIF-1α effects on Claudin-1 (CLDN1) promoter activity. Lastly, HIF-1α-stabilization with the PHD-inhibitor AKB-4924 (1mg/kg-SC) was performed in our L2-IL-5^{OXA}-EoE mouse model.

Results: Here we demonstrated that HIF-signaling is dysregulated in esophageal epithelium following chronic inflammatory-hypoxia resulting in barrier dysfunction and potential disease perpetuation. EPC2-cells exposed to chronic hypoxia resulted in significantly decreased HIF-1α mRNA (70%, $P<0.05$) and decreased target gene induction (GLUT-1, 85%, $P<0.001$) compared to normoxic cells. Activated eosinophils consumed significantly greater oxygen compared to unstimulated cells. Molecular analysis of EoE subject biopsies yielded similar results *ex vivo* (decreased HIF-1α protein 50%, $P<0.001$; HIF-1α mRNA 70%, $P<0.001$; GLUT1 mRNA 85%, $P<0.001$; active inflammation-vs.-control). Concordant with our observations in cells and EoE subject specimens, analysis of our L2-IL-5^{OXA}-EoE mouse model confirmed dysregulated HIF-signalling (decreased mRNA expression HIF-1α, 30%, $P<0.05$; GLUT1, 45%, $P<0.01$; (active inflammation-vs.-control)). Since our work examining both human and mouse epithelia demonstrates significantly altered barrier by electron microscopy, we went on to further examine tight junctions. Molecular analysis of EPC2-cells exposed to chronic hypoxia demonstrated significant decreases in the tight junction associated CLDN1 transcript (60%, $P<0.05$). Consistent with this, array of human and mouse tissues identified a significant decrease in CLDN1 mRNA expression in EoE subject biopsies (80%, $P<0.01$; actively inflamed-vs-control) and our L2-IL-5^{OXA}-mouse model (30% decrease, actively inflamed-vs-control, $P<0.05$). Using *in vitro* 3D-ALI barrier function assays HIF-1α-dominant negative cells exhibited 15% decreased TEER and 1.8-fold increased FITC-flux (DN-vs-control cells, $P<0.05$). This was confirmed with HIF-1α-knockdown-cells that exhibited 25% decreased TEER and 1.9-fold increased FITC-flux. Mechanistically, ChIP and promoter-mutagenesis luciferase studies confirmed HIF-1α binding and activation of the CLDN1 promoter. Lastly, pharmacological HIF-stabilization by AKB-4924 treatment of L2-IL-5^{OXA}-EoE-mice ameliorated disease, causing reduced eosinophilic infiltration (10 vs 28 eosinophils/hpf, $P<0.01$) and epithelial basal zone hyperplasia (decreased 45%, $P<0.05$) (treated-vs-actively inflamed).

Conclusions: Collectively, these studies reveal that HIF-1α de-stabilization in EoE results in dysregulated esophageal epithelial barrier integrity and presents HIF-1α stabilization as a therapeutic target for mucosal healing in EoE.

Grant support: This work was supported by the American Partnership for Eosinophilic Disorders HOPE Pilot Grant (JCM) and a National Institutes of Health NIDDK- K01 grant (JCM).

MICRORNA-155 REGULATES AIRWAY GROUP 2 INNATE LYMPHOID CELLS (ILC2) IN MURINE MODELS OF ALLERGIC AIRWAY INFLAMMATION

Kristina B.C. Johansson[1], Carina Malmhäll1 and Madeleine Rådinger[1]

[1]University of Gothenburg, Sahlgrenska Academy, Department of Internal Medicine and Clinical Nutrition, Krefting Research Centre, Gothenburg, Sweden

Background: Allergic airway inflammation (AAI) is triggered by allergen exposure through several steps including release of epithelial-derived cytokines such as interleukin (IL)-33. IL-33 promotes TH2 cytokine (IL-13, IL-5) release from group 2 innate lymphoid cells (ILC2) leading to TH2 cell differentiation and further production of TH2 cytokines IL-4, IL-5 and IL-13. We have previously demonstrated that microRNA (miR)-155 deficient mice fail to develop a proper TH2 response and airway eosinophilia in an acute mouse model of ovalbumin (OVA)-induced AAI. The molecular mechanisms leading to the release of epithelial-derived cytokines and an abnormal TH2 response remain largely unclear. Importantly, the role of miRs in the regulation of these processes including allergen-induced ILC2 expansion is still unexplored.

Aim: The aim of this study was to determine the role of miR-155 in the regulation of ILC2 cellular expansion during AAI using experimental murine models.

Method: Wild-type (WT) and miR-155 deficient (miR-155 KO) C57BL/6J mice were sensitized by intraperitoneal injection (OVA) day 0 and 6 or by intranasal exposure to house dust mite (HDM) on day 1, 2 and 3. To study the acute inflammatory response mice received 5 (OVA) or 4 (HDM) intranasal exposures before being sacrificed 24 hours after the final exposure. In order to mimic a chronic inflammatory condition, 14 days post OVA sensitization mice received OVA intranasally 3 times a week for 12 weeks. AAI was also induced by intranasal exposures with recombinant murine IL-33 on day 0, 2 and 4 before being sacrificed on day 5. Non-allergic control mice were exposed to PBS. Lung ILC2 cells were quantified by flow cytometric analysis and lung IL-33 was measured by ELISA.

Results: WT mice demonstrated an increase of ILC2 cells locally in the lung upon IL-33 treatment ($p<0.05$). In contrast, this was not seen in miR-155 KO mice where the ILC2 cell population remained at the level of PBS exposed controls. Following acute or chronic OVA challenge ILC2 cells increased in WT but not in miR-155 KO mice. However, lung ILC2 cell numbers remained unchanged upon acute HDM challenge. IL-33 expression in lung tissue was shown to increase in allergen-challenged WT mice but not miR-155 KO mice ($p<0.05$).

Conclusion: The impaired IL-33 expression in miR-155 deficient mouse lung indicates that miR-155 might in addition to direct ILC2 cellular function be important for epithelial function. This could possibly in part explain the observation that miR-155 deficient mice demonstrate impaired eosinophilic airway inflammation, a hallmark of AAI. Our findings, for the first time, suggest that ILC2 cell expansion might be dependent on miR-155.

EOSINOPHILS PROMOTE COLORECTAL CANCER THROUGH EXPRESSION OF S100A8 AND S100A9.

Reichman Hadar [1], Itan Michal [1], Varol Chen [2], Pasmanik-Chor Metsada [3], Munitz Ariel[1]

[1] Department of Clinical Microbiology and Immunology, the Sackler School of Medicine, Tel-Aviv University, Ramat Aviv 69978, Israel, [2] Research Center for Digestive Tract and Liver Diseases, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 64239, [3] Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv 69978, Israel.

Background: Eosinophils are bone marrow-derived cells that have been largely implicated in Th2-associated diseases. However, recent data highlight key roles for eosinophils in non-classical Th2 settings (e.g metabolism, thermogenesis and tissue regeneration). Quite surprisingly despite the fact that the gastrointestinal (GI) tract is one of the largest eosinophil reservoirs in the body, the roles of GI eosinophils have been largely understudied especially in chronic GI inflammation and subsequent tumorigenesis.

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: Substantial eosinophilic infiltration was observed in all three models of murine CRC. Eosinophil recruitment was accompanied with a significant increase in CCL11 and with decreased blood eosinophilia, suggesting active recruitment of eosinophils to the colon. AOM+DSS-treated eosinophil-deficient mice (ΔdblGATA mice) displayed decreased epithelial cell proliferation, decreased collagen deposition and decreased expression of matrix metaloproteinases, tissue inhibitors of matrix metalloproteinases

and TNF-α. Consequently, tumor load was dramatically reduced in the colons of AOM+DSS-treated and orthotopically transplanted ΔdblGATA mice.

Microarray analysis of primary colonic eosinophils that were sorted at various stages of the tumorigenic process (naïve-, inflammatory-, tissue repair- and tumor associated-eosinophils) revealed dynamic regulation of colonic eosinophil mRNA expression during carcinogenesis. Interestingly, the pro-tumorigenic and clinically relevant genes s100a8 and s100a9 were strikingly and kinetically increased in colonic eosinophils (250-fold and 90-fold, respectively). Indeed, local and systemic expression of s100a8 and s100a9 were nearly diminished in AOM+DSS-treated ΔdblGATA mice, and were re-constituted upon adoptive transfer of eosinophils into the colon.

Conclusions: We now report that eosinophils are a bona-fide cellular compartment of the tumor microenvironment in CRC. These data establish a key and unforeseen pro-tumorigenic role for eosinophils in CRC. Furthermore, our results suggest that eosinophils can promote carcinogenesis via expression and secretion of s100a8 and s100a9.

Grant support: Israel Cancer Association; Israel Cancer Research Foundation.

SIMILAR PHENOTYPIC CHANGES ON EOSINOPHILS IN DIFFERENT LUNG COMPARTMENTS DURING NORMAL POSTNATAL LUNG DEVELOPMENT AND ALLERGIC INFLAMMATION SUGGEST THEIR HOMEOSTATIC FUNCTION IN THE AIRWAY MUCOSA

Lucas F. Loffredo[1], Hiam Abdala-Valencia[1] and Sergejs Berdnikovs[1]

[1]Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine

Objective: Eosinophil recruitment to the lung is a multi-step process. While the trans-endothelial step is well studied, reasons for trans-epithelial eosinophil migration are much less understood. Moreover, phenotypic and functional differences in eosinophils between these two compartments are not yet characterized. The objective of this study was to determine whether eosinophils exhibit phenotypic plasticity based on their recruitment to different lung compartments, and to gain new clues to why and how eosinophils interact with the airway mucosa. During this study, we unexpectedly identified a wave of lung eosinophilia peaking at day 10 postnatal development, which led us to compare eosinophil recruitment between allergic inflammation and normal development.

Methods: We quantified and phenotyped eosinophils recruited to the lung during normal postnatal development or allergic inflammation by multi-color flow cytometry, simultaneously detecting these cells in bronchoalveolar lavages (BAL) and digested lung tissue. For allergic inflammation, we employed a kinetic murine model of ovalbumin (OVA)-induced asthma with two OVA/alum sensitizations and six consecutive OVA challenges, harvesting tissues at 6 hours after each challenge. Postnatal lung development was assessed in wild type mice at days 3, 6, 8, 10, 13, 21 and adulthood. Additionally, we measured gene expression of epithelial differentiation and repair markers in digested lung tissue.

Results: We identified two distinct populations of eosinophils recruited to the lung tissue of OVA-challenged mice: Siglec-F^{medium}CD11c^{-} and Siglec-F^{high}CD11c^{low}. Upregulation of lectin Siglec-F and integrin CD11c by eosinophils in lung tissue became more pronounced with more challenges, which coincided with more dominant eosinophil presence in the BAL. BAL-recruited eosinophils only exhibited one eosinophil phenotype (Siglec-F^{high}CD11c^{low}), suggesting that only this population crosses the epithelial barrier and enters the airways. Concomitantly, CD11c^{+} eosinophils demonstrated gene expression of specific cytokine/chemokine receptors IL1RL1, IL1RL2, IL1R2, CCR1, CCR2 and CCR5, integrins Itgae, Itgam, Itgb1 and Itgb5, and markers CD2, CD36 and CD47, all indicating enhanced capacity for mucosal docking/interaction. Remarkably, we also have detected previously undocumented recruitment of eosinophils to the lung in normal postnatal development beginning on day 6 and peaking on day 10 at ~20%, which perfectly synchronized with alveolarization and epithelial differentiation processes in the developing lung. Analysis of the lung tissue itself, in both development and allergic inflammation, revealed that presence of eosinophils correlated with expression of markers of epithelial differentiation/repair (Adam33, Fbn1, Nes, Tnc, Wnt5a), supporting attraction of eosinophils to mucosal sites undergoing remodeling.

Conclusions: Upregulation of Siglec-F and CD11c on eosinophils recruited to the interstitium of both developing and allergen-challenged mouse lung, and evidence that all airway eosinophils belong to Siglec-F^{high}CD11c^{low} phenotype, suggest the existence of a specific homeostatic mechanism targeting these cells to the airway mucosa. Synchronization of eosinophil recruitment with tissue expression of markers of epithelial remodeling in both normal development and allergic inflammation suggests that tissue eosinophils are specifically targeted to mucosal sites with increased need for differentiation or repair. Collectively, our results suggest that eosinophils are specifically attracted to pulmonary sites of mucosal remodeling in vivo.

EFFICACY, SAFETY, AND PATIENT-REPORTED OUTCOMES WITH RESLIZUMAB IN PATIENTS WITH ASTHMA AND ELEVATED BLOOD EOSINOPHILS: A RANDOMIZED PHASE 3 STUDY

Leif Bjermer, Catherine Lemiere, Jorge Maspero, Sivan Weiss, Matthew Germinaro and James Zangrilli

Background: Reslizumab, a humanized anti-interleukin-5 monoclonal antibody, is in development for patients with uncontrolled moderate-to-severe persistent asthma with elevated blood eosinophils. Here, we report the short-term efficacy, safety, and patient-reported outcomes of reslizumab versus placebo in this patient population.

Methods: This phase 3, multicenter, placebo-controlled, double-blind, 16-week study (NCT01270464) included 311 patients (12–75 years of age) with blood eosinophil levels ≥400 cells/μL and uncontrolled asthma based on an Asthma Control Questionnaire (ACQ) score ≥1.5 while receiving at least medium-dose inhaled corticosteroids (equivalent to fluticasone ≥440 μg daily). Randomization was to intravenous reslizumab 0.3 mg/kg (n=104), or 3.0 mg/kg (n=106), or placebo (n=105) once every 4 weeks. Efficacy variables included pre-bronchodilator pulmonary function (forced expiratory volume in 1 second [FEV1]; primary variable) and various patient-reported outcomes including ACQ scores (7-point scale, higher scores indicate increasing impairment; minimal important difference [MID] 0.5 U), Asthma Quality of Life Questionnaire (AQLQ; 7-point scale, higher scores indicate better quality of life; MID 0.5 U), and Asthma Symptom Utility Index (ASUI; 0 [worst possible symptoms]; 1 [no symptoms]).

Results: Following 16 weeks of therapy, reslizumab significantly improved the overall FEV1 and ACQ score (treatment difference vs placebo [reslizumab 0.3 and 3.0 mg/kg respectively]: FEV1, 115 and 160 mL [p≤0.024]; ACQ, 0.238 and 0.359 [p≤0.033]). Improvements in the reslizumab 3.0 mg/kg treatment group were observed in both measures (FEV1, 153 mL; ACQ, –0.283 [p≤0.015]) at 4 weeks and maintained throughout the treatment period. Clinically meaningful improvements over 16 weeks in forced vital capacity (130 mL, p=0.017) and forced expiratory flow 25–75% (0.233 L/s; p=0.055) were observed for the reslizumab 3.0 mg/kg treatment group, but not for the 0.3 mg/kg group (48 mL and 0.030 L/s, p>0.2 for both, respectively). At week 16, reslizumab 0.3 mg/kg and 3.0 mg/kg improved AQLQ score from baseline (mean treatment difference vs placebo: 0.278 [p=0.082] and 0.359 [p=0.024], respectively). The percentage of patients with an MID in AQLQ at week 16 were: 59% [p=0.082] for the reslizumab 0.3 mg/kg treatment group, 64% [p=0.019], for the reslizumab 3.0 mg/kg treatment group, and 48% for the placebo group. Improvements in ASUI were observed in both treatment groups (mean treatment difference vs placebo: 0.051, reslizumab 0.3 mg/kg [p=0.009]; 0.047, reslizumab 3.0 mg/kg [p≤0.016]). Decreases in blood eosinophils for the 3.0 mg/kg and the 0.3 mg/kg dose levels were 494 cells/μL and 323 cells/μL, respectively. The most commonly reported adverse events (AEs) were asthma, headache, nasopharyngitis, bronchitis, and upper respiratory tract infection. AEs led to discontinuation in <1%, 6%, and 10% of patients in the reslizumab 0.3 mg/kg treatment group, reslizumab 3.0 mg/kg treatment group, and placebo group, respectively.

Conclusions: In patients with uncontrolled asthma and elevated blood eosinophils, four (monthly) doses of reslizumab at 0.3 and 3.0 mg/kg were well-tolerated and resulted in improvement in multiple measures of asthma control. The 3.0 mg/kg dose level generally produced larger treatment effects, particularly on indices of small airway function.

Grant support: This study was sponsored by Teva Pharmaceuticals.

DEVELOPMENT OF A NOVEL PEPTIDE NANOPARTICLE-BASED ANTAGONIST OF HUMAN CCR3-MEDIATED EOSINOPHIL MIGRATION IN ALLERGIC INFLAMMATION

Kimberly G. Laffey[1]; Boris Garnier[1]; Ben Hitchinson[1]; Nadya Tarasova[2]; Vadim Gaponenko[1] and Steven J. Ackerman[1].

[1]Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Illinois USA; [2]Center for Cancer Research, National Cancer Institute, Maryland, USA

Background: Eosinophils constitute the major cell type in the inflammatory infiltrate in eosinophilic asthma. CCR3 signaling is a major mechanism through which eosinophils are recruited to the site of inflammation where they are involved in mediating inflammation, tissue damage and remodeling. CCR3 is also expressed by several other inflammatory cell types and binds promiscuously to many chemokines. Inhibiting CCR3 signaling is therefore a promising therapeutic strategy. However, no CCR3 inhibitor has been approved for clinical use to date. We sought to develop novel CCR3 peptide nanoparticle- based biased antagonists that target the receptor to reduce chemotaxis and recruitment of multiple immune cells in allergic inflammation.

Methods: We designed peptides containing transmembrane domains with and without extracellular loop portions of CCR3 known to interact with its chemokine ligands. Peptide binding to CCR3 was analyzed by NMR. The inhibitory effect on CCR3 function was evaluated using CCR3-mediated chemotaxis and signaling induced by CCL11 (eotaxin-1), CCL5 (RANTES) and CCL28 (MEC) ligands in CCR3+ cell lines (AML14.3D10-CCR3) and purified blood eosinophils from allergic subjects with mild asthma.

Results: A peptide inhibitor containing transmembrane and extracellular loop portions of CCR3 (termed R3-2-1) forms nanoparticles and directly binds to the receptor. It inhibits both CCR3-mediated chemotaxis and signaling at low micromolar concentra-

tions, at levels comparable to small molecule CCR3 antagonists. These effects are mediated in part through enhancing chemokine ligand-induced CCR3 endocytosis and attenuation of intracellular signaling through the MAPK and PI3K pathways.

Conclusions: We have identified a peptide, R3-2-1, that potently inhibits CCR3-mediated eosinophil chemotaxis induced by multiple chemokine ligands. The peptide forms nanoparticles that stabilize and protect it from proteolytic degradation. The peptide likely disrupts receptor conformation allosterically, inhibiting downstream signaling but not ligand-induced endocytosis. This represents a unique mode of action (biased antagonism) compared to small molecule CCR3 competitive inhibitors that are being tested in clinical trials, and may provide a novel therapeutic approach to asthma and other eosinophil- associated allergic and idiopathic diseases. Understanding peptide-based inhibition should also provide valuable insights into the mechanisms of CCR3 signaling.

Grant support: Supported by NIH/NHLBI Grant R21HL118588 (to VG and SJA), and by NIH/NCI Grants R01 CA135341 and R01 CA188427 (to VG).

POSTER 1

PERIPHERAL B CELLS AND EOSINOPHILS ARE POSITIVELY CORRELATED IN UNTREATED HUMAN SUBJECTS WITH EOSINOPHILIA

Fei Li Kuang[1], Shakuntala Rampertaap[2], Nicole Holland-Thomas[3], Paneez Khoury[1], and Amy D. Klion[1]

[1] Human Eosinophil Section, Laboratory of Parasitic Disease, NIAID, National Institutes of Health, Bethesda, MD, [2] Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, MD, [3] Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

Background: With the advent of novel targeted therapies that dramatically reduce tissue and blood eosinophilia, understanding the role of eosinophils in homeostasis is becoming increasingly important. Although recent studies in murine models suggest that eosinophils are important in the maintenance of B cell numbers and function, extrapolation to humans is complicated by the fact that IL5 receptor is expressed on murine but not human B cells. A small, but statistically significant, positive correlation between absolute eosinophil count (AEC) and peripheral B cell count was recently reported in a retrospective analysis of normal and atopic individuals as well as subjects with hypereosinophilic syndrome (HES). However, clinical information, including treatment status and other potentially confounding factors, was not provided.

Methods: To further explore the relationship between eosinophils and B cells in the setting of eosinophilia, a retrospective analysis of clinical and laboratory data from 373 consecutive subjects referred for evaluation of unexplained eosinophilia between 1994 and 2015 was performed. Adult subjects with AEC ≥ 1000/mm^3, on no therapy for eosinophilia, and for whom whole blood flow cytometry results were available, were selected for analysis (n=107). B cells were identified in the lymphocyte gate on the basis of CD19 and/or CD20 surface expression. Eosinophil surface expression of CD69 was assessed as a marker of eosinophil activation. Correlations and comparison of group means were performed using non-parametric statistical methods.

Results: Of the 107 subjects included in the analysis, 23 had documented secondary causes of eosinophilia, such as helminth infection (n=8) and neoplasia (n=3). The remaining 84 subjects were diagnosed with HES. AEC was positively correlated with peripheral B cell count in the entire cohort (r=0.225 p=0.02) and in the subjects with HES (r =0.23 p=0.03) but not those with secondary eosinophilia (r=0.10, p=0.64). AEC was also correlated with serum IgG and IgM levels in the entire cohort (r=0.26, p<0.01 and r=0.226, p=0.02, respectively) and in the subjects with HES (r=0.255, p=0.02 and r=0.243, p=0.03 respectively), but not in subjects with secondary eosinophilia.

To begin to explore the role of the etiology of the eosinophilia on B cell numbers, subjects with myeloproliferative HES (MHES, n=12) were compared to those with lymphocytic variant HES (LHES, n=10). Whereas AEC was strongly correlated with peripheral B cell count in MHES subjects (r=0.615 p=0.04), there was a negative, albeit non-significant, correlation observed in LHES subjects (r =-0.442, p=0.20). Of note, the MHES subjects had significantly increased AECs compared to the LHES subjects (geometric mean 8212 vs. 3391, Mann-Whitney p<0.01) as well as a trend towards increased eosinophil activation, as assessed by eosinophil CD69 expression (GM 14.8% vs 3.8%, Mann-Whitney p=0.11).

Conclusions: These data confirm a positive correlation between AEC and peripheral B cell count in untreated subjects with HES and suggest that this effect may be enhanced in the setting of eosinophil activation. Whether prolonged and profound depletion of eosinophils through the use of targeted therapies will lead to reduced B cell numbers or function remains to be seen.

Grant support: This study was funded by the Division of Intramural Research, NIAID, NIH and by NCI Contract No. HHSN261200800001E. This research was supported in part by the National Institute of Allergy and Infectious Diseases.

POSTER 2

IDENTIFICATION OF THE TRANSCRIPTION FACTOR REPERTOIRE DURING HOMEOSTATIC EOSINOPHILOPOIESIS

Carine Bouffi[1], Andrey V. Kartashov[1], Kaila L. Schollaert[1], Xiaoting Chen[4], W. Clark Bacon[1], Matthew T. Weirauch[2], Artem Barski[1,3], and Patricia C. Fulkerson[1]

[1]Division of Allergy and Immunology, [2]Center for Autoimmune Genomics and Etiology, [2]Division of Biomedical Informatics and Division of Developmental Biology, [3]Division of Human Genetics, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229; [2]School of Electronic and Computing Systems, University of Cincinnati, Cincinnati, OH 45221.

Background: The production of mature eosinophils is a tightly orchestrated process with the aim to sustain normal eosinophil levels in tissues, while maintaining low numbers of these complex and sensitive cells in the blood. Murine studies with genetically altered animals provide a wealth of evidence supporting not only a critical role for IL-5 in mediating disease-associated eosinophilia,

but also, unexpectedly, that IL-5 is not required for baseline eosinophil production. The regulatory molecules or networks necessary for optimal homeostatic eosinophil production remain largely unknown.

Methods: To identify regulators of homeostatic eosinophilopoiesis in mice, we took a global approach to identify genome-wide transcriptome and epigenome changes that occur during homeostasis at critical developmental stages, including eosinophil-lineage commitment (eosinophil progenitor [EoP] compared to granulocyte-monocyte progenitor [GMP]) and lineage maturation (eosinophil compared to EoP). We performed chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-seq) and RNA sequencing (RNA-seq) to identify transcription factors (TFs) and genetic regulatory elements that are active during homeostatic eosinophil production in the bone marrow.

Results: Our analyses revealed markedly greater transcriptome alterations associated with eosinophil maturation (1199 genes) compared to eosinophil-lineage commitment (490 genes), highlighting the greater transcriptional investment necessary for differentiation. EoPs were noted to express high levels of granule proteins and contain granules with an ultrastructure distinct from mature resting eosinophils. Our analyses also delineated a 976 gene eosinophil-lineage transcriptome that included a repertoire of 56 transcription factors, many of which have never previously been associated with eosinophils. EoPs and eosinophils, but not GMPs or neutrophils, expressed Helios and Aiolos, members of the Ikaros family of transcription factors that regulate gene expression via modulation of chromatin structure and DNA accessibility. In addition, Aiolos and Helios binding sites were significantly enriched in genes expressed by EoPs and eosinophils with active chromatin.

Conclusions: Collectively, our study reveals that the dynamic changes in gene expression associated with eosinophil development include novel transcriptional regulators, such as Helios and Aiolos, and distinct epigenetic profiles between EoPs and eosinophils. Comprehensive epigenomic and transcriptomic profiling during critical stages in eosinophil development will ultimately define the programming and gene regulatory networks necessary for eosinophil development and will likely lead to novel therapeutic strategies to regulate eosinophil production. In addition, our study highlights that the regulatory mechanisms that direct eosinophil homeostasis are likely to be developmental-stage (EoP vs. eosinophil) specific. These findings have implications for a number of diseases, including allergic and eosinophilia-associated disorders, in which these processes may be manipulated for therapeutic benefit.

Grant support: This work was supported by the NIH grant K08 AI093673 and the Allergy, Asthma, Immunology Education and Research Organization (ARTrust) Faculty Development Award of the American Academy of Allergy, Asthma and Immunology. This project was also supported in part by the NIH grant P30 DK078392 (Gene and Protein Expression and Flow Cytometry Cores) of the Digestive Disease Research Core Center in Cincinnati.

POSTER 3

TNF MEDIATES HEMATOPOESIS OF BENIFICAL EOSINOPHILS THREE DAYS AFTER OZONE

Sarah A. Wicher, David B. Jacoby, Allison D. Fryer

Division of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR 97239

Background: Ozone is an urban air pollutant that temporarily reduces lung function in healthy individuals, but in asthmatics, exacerbates disease and increases hospitalizations. In guinea pigs ozone causes airway hyperreactivity that lasts at least three days. Ozone additionally recruits inflammatory cells, including eosinophils, to lungs. One-day after ozone, airway hyperreactivity is mediated by eosinophils that increase acetylcholine release from parasympathetic nerves resulting in increased bronchoconstriction (AJP. 287:627). In contrast, three days after ozone eosinophils have acquired an unexpected protective role since depleting them with an antibody to IL5 makes airway hyperreactivity significantly worse. However, in sensitized guinea pigs the beneficial role of eosinophils is lost. Since one mechanism of eosinophil recruitment is tumor necrosis factor-α (TNF) (JI. 158:954), we tested whether TNF plays a role in ozone-induced eosinophil hematopoiesis, recruitment of beneficial eosinophils to lungs, and airway hyperreactivity in non-sensitized and sensitized guinea pigs.

Methods: Guinea pigs were sensitized to ovalbumin (4.2 mg i.p. every other day for 3 days). 21 days later, both sensitized and non-sensitized guinea pigs were treated with 5-bromo-2'-deoxyuridine (BrdU, 100mg/kg i.p.) the day of exposure to air or ozone (2.0ppm, 4h) and daily thereafter (50mg/kg i.p.) to label newly divided cells. Three hours prior ozone animals were pretreated with the TNF antagonist etanercept (3mg/kg i.p.). Three days after ozone guinea pigs were anesthetized with 1.9g/kg urethane i.p., paralyzed with succinylcholine i.v., and ventilated at constant volume and flow. Both vagi were cut and placed on platinum electrodes connected to a stimulator. Bronchoconstriction was measured as an increase in pulmonary inflation pressure (mmH20) in response to electrical stimulation of the vagus nerves (2-25hz, 10V, 0.2msec 5sec on 60sec off). Inflammatory cells were harvested from bone marrow, blood, and lungs.

Results: In non-sensitized guinea pigs ozone caused airway hyperreactivity, increased eosinophil hematopoiesis in bone marrow, and increased recruitment of newly divided eosinophils to lungs. Sensitization significantly increased ozone induced airway hyper-

reactivity. However, in sensitized guinea pigs ozone increased eosinophils in lungs, but did not increase newly divided eosinophils in lung or bone marrow. Thus, in the absence of newly divided eosinophils, airway hyperreactivity was potentiated. Similarly, in non-sensitized animals, etanercept potentiated ozone-induced airway hyperreactivity. Furthermore, etanercept increased eosinophils in the lungs but inhibited ozone induced eosinophil hematopoiesis and recruitment of newly divided cells to lungs. Thus, in the absence of TNF airway hyperreactivity was potentiated, but ozone induced eosinophil hematopoiesis was inhibited similar to what was observed in sensitized animals. In contrast etanercept had no additional effect on ozone-induced airway hyperreactivity or eosinophil hematopoiesis in sensitized guinea pigs.

Conclusion: These data demonstrate a novel protective role for eosinophils three days after ozone. The protective role appears to be due to the presence of newly divided eosinophils since either sensitized or etanercept potentiated ozone induced airway hyperreactivity, while specifically blocking eosinophil hematopoiesis and recruitment of newly divided eosinophils to lungs. In non-sensitized guinea pigs ozone induced eosinophil hematopoiesis is mediated by TNF.

Grant support: Health Effects Institute (ADF), ES017593 (ADF), HL113023 (DBJ), AR061567 (DBJ), HL124165 (DBJ)

POSTER 4

EOSINOPHIL PROGENITOR MOBILIZATION IN ACTIVE EOSINOPHILIC ESOPHAGITIS

David W. Morris[1], Emily M. Stucke[1], Marc E. Rothenberg[1], MD, PhD, Patricia C. Fulkerson[1], MD, PhD

[1]Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229.

Background: Eosinophils develop in the bone marrow from an eosinophil-lineage committed progenitor (EoP) that expresses CD34 and the receptor for IL-5 (IL-5R-alpha or CD125). EoPs are increased in the bone marrow, peripheral blood and lung tissue of asthmatic patients after allergen challenge. EoPs have been implicated in contributing to disease pathogenesis through in situ proliferation and differentiation in inflamed tissues. Notably, EoP levels in the peripheral blood correlate with asthma severity in adults. The role of EoPs in eosinophilic gastrointestinal disorders, such as eosinophilic esophagitis (EoE), has not been determined. Furthermore, the frequency of EoPs in the peripheral blood of pediatric patients has not been reported. We hypothesize that the frequency of EoPs in the peripheral blood will directly correlate to the number of mature eosinophils in the esophagus of pediatric patients with EoE.

Methods: Patients ages 1-18 who were undergoing endoscopy for EoE management were recruited through the Cincinnati Center for Eosinophilic Disorders. Patients were free of other gastrointestinal inflammatory diseases or systemic corticosteroids within the last two months. Peripheral blood samples were obtained at the time of esophageal biopsy. EoPs were identified in blood samples by flow cytometry ($CD34^+$, $CD38^+$, $CD125^+$, $CD45RA^-$). EoP levels were compared with peak eosinophil counts in the esophageal biopsy samples. We expect to recruit a minimum of 42 patients. Preliminary results are presented for 23 consented participants with EoE; non-active (n=11, <15 esophageal eosinophils/HPF and active (n=12, >15 esophageal eosinophils/HPF).

Results: Our preliminary findings reveal that EoP frequency was elevated in the blood of pediatric patients with active EoE compared to pediatric patients with inactive disease (Non-Active: 16.45 +/- 3.72 EoPs/mL vs. Active: 31.75 +/- 6.75 EoPs/mL, $P = 0.0665$). EoP levels positively correlated with esophageal eosinophils (Spearman $r = 0.44$, $P = 0.036$). There were no significant differences in EoP frequency between groups based on gender, age or atopic status.

Conclusions: EoPs are increased in the peripheral blood of pediatric patients with active EoE compared to pediatric patients with inactive disease and positively correlate with esophageal eosinophils.

Grant support: This work was supported in part by NIH R37 AI045898, U19 AI070235, U19 AI066738 (CoFAR supported by NIAID and NIDDK), R01 DK076893, R01 AI057803**,** P30 DK078392 (Gene and Protein Expression Core), U54 AI117804 (CEGIR supported by NCATS, NIAID and NIDDK), the Campaign Urging Research for Eosinophilic Disease (CURED), the Buckeye Foundation, and Sunshine Charitable Foundation" and its supporters, Denise A. Bunning and David G. Bunning. Additionally, this work was supported by an ARTrust Mini Grant (D.W.M.), an ARTrust Faculty Development Award (P.C.F.), and by the NIH grant K08 AI093673 (P.C.F.). This project was also supported in part by the NIH grant P30 DK078392 (Flow Cytometry Core) of the Digestive Disease Research Core Center in Cincinnati.

POSTER 5

AGE AND GENDER DEMOGRAPHIC ANALYSIS OF CASES REFERRED FOR INVESTIGATION OF FIP1L1-PDGFRA POSITIVE CHRONIC EOSINOPHILIC LEUKEMIA IN AUSTRALIA

Alberto Catalano, Francisca Springall, Dimitrii Minchenko, Cheryl Paul, Shane G. Supple, and Harry Iland

Institute of Haematology, Royal Prince Alfred Hospital, Camperdown NSW 2050, Australia

Background: Since 2004, our laboratory has been testing for the presence of the *FIP1L1-PDGFRA* gene rearrangement in referred samples from patients with hypereosinophilia being investigated for the presence of the *FIP1L1-PDGFRA* gene rearrangement. *FIP1L1-PDGFRA* positive chronic eosinophilic leukemia (CEL) occurs almost exclusively in males with only few reported female cases. We sought to determine whether this male bias also exists in Australia and to analyse age and sex demographics in referred patients as a surrogate cohort of hypereosinophilia in the Australian population.

Methods: *FIP1L1-PDGFRA* fusion transcripts were detected by reverse transcription and polymerase chain reaction. De-identified data including presence of *FIP1L1-PDGFRA*, gender, age at first referral and geographic origin were collected from all specimens referred to our laboratory from 2004 until the end of April 2015 from throughout Australia. The patient age distributions in 10-year age groupings were compared using the chi-squared test and using demographic data of the general Australian population (Australian Bureau of Statistics, 2010). Incidence of *FIP1L1-PDGFRA* by gender was compared using Fisher's exact test.

Results: The patient cohort consisted of 1079 patients referred from all Australian states and territories. There were 599 males (56%) and 480 females (44%) with respective median ages of 61 (range 6-95) and 54 (range 6-93) years. The age distributions of the referred male and female cohorts were significantly different to each other ($p<0.0001$) and to the respective Australian male ($p<0.0001$; median age 36 years) and female ($p<0.0001$; median age 37 years) populations. Peak incidences of hypereosinophilia within the cohort were in age groups 70-79 in males and 50-59 in females. The annual age adjusted incidence of hypereosinophilia increases with age and is similar between males and females upto the age group of 50-59; in all 10-year age groupings above 60, the age adjusted incidence of hypereosinophilia is higher in males than females.

There were 25 male and 1 female *FIP1L1-PDGFRA* positive patients in the cohort with median ages of 53 (range 14-74) and 29 (single case) years, respectively. The incidence of *FIP1L1-PDGFRA* positive CEL was 2.4% of all patients tested and the male gender bias (96%) was significant ($p<0.0001$).

Conclusions: When investigating patients with hypereosinophilia, causes other than CEL are likely to be important. In particular, females with *FIP1L1-PDGFRA* positive CEL are extremely rare. Differences in the age distributions of the male and female patient cohorts suggest that hormonal factors may be involved in the aetiology of hypereosinophilia. However, the extreme male gender bias in the incidence of *FIP1L1-PDGFRA* positive CEL observed in this cohort and in other reported cohorts, by other investigators, is suggestive of involvement of a recessive sex-linked predisposition or cooperative gene mutation in the etiology of CEL.

POSTER 6

MATERNAL SUPPLEMENTATION OF ALLERGIC FEMALE MICE WITH GAMMA-TOCOPHEROL INCREASES THE DEVELOPMENT OF SELECT DENDRITIC CELL SUBSETS AND ALLERGIC LUNG INFLAMMATION IN NEONATES.

Joan Cook-Mills, Hiam Abdala-Valencia, and Frank Soveg

Allergy/Immunology Division, Northwestern Univ. School of Medicine, Chicago, IL

Background: Offspring from allergic mothers have elevated numbers of CD11b+ lung dendritic cells and develop allergic lung responses to suboptimal allergen challenge. We recently reported that maternal supplementation with alpha-tocopherol (αT) reduced development of these dendritic cell subsets and allergic responses in offspring of allergic female mice. It is not known whether gamma-tocopherol (γT) supplementation regulates development of allergic responses.

Methods: To address this, allergic female mice were supplemented with γT during pregnancy/lactation. Then, offspring were given a suboptimal allergen challenge.

Results: γT supplementation of allergic mothers elevated pup responses to allergen challenge. There were increased numbers of pup lung eosinophils, inflammatory mediators, and CD11b+ but not CD11b- subsets of CD11c+ dendritic cells. There were

elevated numbers of IRF4+ CD11b+ CD11c+ dendritic cells, a dendritic cell subset critical for development of allergic responses. There were also fewer pups from γT supplemented allergic mothers.

Conclusion: Maternal αT supplementation reduced and maternal γT supplementation increased development of CD11b+ CD11c+ dendritic cells and allergic responses in offspring from allergic mothers. These results have implications for supplementation of allergic mothers with tocopherol isoforms and for development of allergies in future generations.

Grant support: NIH R01HL111624

POSTER 7

EXOSOMES SECRETION BY EOSINOPHILS: A POSSIBLE ROLE IN ASTHMA PATHOGENESIS

Carla Mazzeo, José Antonio Cañas, Mª Paz Zafra, Joaquín Sastre, Victoria Del Pozo

Background: Eosinophils secrete cytotoxic granules which are involved in initiation and propagation of diverse inflammatory responses as asthma. Our hypothesis is that some of these granules are exosomes which contain miRNAs and participate in the pathogenesis of these diseases. Our aims are to characterize the eosinophils exosomes and to investigate their role in asthma.

Methods: We study the capacity of eosinophils to generate intracellular precursors of exosomes (multivesicular bodies, MVBs) by electron, confocal and fluorescence microscopy and flow cytometry analysis. Eosinophils were labelled with the specific marker of MVBs (Lysobiphosphatidic Acid, LBPA) and the reporter of endosomal vesicles (CD63). Exosomes derived from eosinophils were characterized by Western Blot (WB), Nanoparticle Tracking Analysis (NTA) to estimate the size distribution and concentration, flow cytometry and by electron microscopy images.

Results: We observed that eosinophils generate intracellular MVBs. It was confirmed by the colocalization of LBPA and CD63 in these vesicles. This result was corroborated by electron microscopy images. Exosomes purified from eosinophils from healthy and asthmatic subjects were CD63+. The amount of exosomes was increased after stimulation with IFN-γ. NTA showed that the size of eosinophils exosomes was into the characteristic exosomal range. Finally, we found that the exosomes production from asthmatic subjects was higher than healthy subjects.

Conclusion: Our findings provide the first evidence that eosinophils produce MVBs and secrete exosomes. It is possible that they have an important implication in the pathogenesis of asthma. Moreover, they also could be considered as a future biomarker.

Grant support: This study was supported by Fondo de Investigación Sanitaria – FIS [PS09/00153 and PI12/00691]; and CIBER de Enfermedades Respiratorias (CIBERES), a Carlos III Institute of Health initiative.

POSTER 9

OSTEOPONTIN BINDS AND MODULATE FUNCTIONS OF EOSINOPHIL-RECRUITING CHEMOKINES

Anele Gela[1], Gopinath Kasetty[1], Matthias Mörgelin[2], Anders Bergqvist[1], Jonas Erjefält[1], James E. Pease[3], Arne Egesten[1]

Presence of eosinophils in the airways is a hallmark of the Th2 response seen in allergic asthma. In addition to airway obstruction, there is also an increased risk of airway infection caused by *Streptococcus pneumonia,* in particular during severe asthma. The latter is also characterized by high levels of the glycoprotein osteopontin (OPN) in the airways. Eosinophils can be recruited to the airways by chemokines including eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, RANTES/CCL5, and MEC/CCL28 that activate the receptor CCR3 on these cells. In addition to inducing chemotaxis, several of these molecules have defensin-like antibacterial properties. We found that OPN bound all eosinophil-recruiting chemokines with high affinity. However, the binding did not affect their CCR3-activating properties nor did administration of OPN in the airways of OVA-sensitized affected airway eosinophilia. The eosinophil-recruiting chemokines all displayed bactericidal activity against the common airway pathogen *S. pneumoniae*, but only CCL26 and CCL28 retained high antibacterial activity in the presence of sodium chloride at physiologic concentrations (140 mM). Preincubation of the chemokines with OPN at equimolar amounts strongly inhibited the antibacterial activity against *S. pneumonia* but the effect of receptor activating activity was negligible. Taken together, the results suggest that OPN impairs host defense activities of the chemokines without affecting their eosinophil-recruiting properties, resulting in increased vulnerability to acquire pneumococcal infection in parallel with sustained allergic inflammation.

POSTER 10

HUMAN EOSINOPHILS RELEASE EXTRACELLULAR DNA TRAPS IN RESPONSE TO *ASPERGILLUS FUMIGATUS*

Valdirene S. Muniz[1]; Mariana S. R. Bica[1], Yasmim A. V. Braga[1]; Rodrigo T. Figueiredo[2], Josiane S. Neves[1]

[1]Institute of Biomedical Sciences,[2]Institute of Biomedical Sciences/Unit of Xerem, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Background: Eosinophils mediate the immune response in a number of infectious diseases, including parasitic helminth, bacterial, fungal and viral infections. The process of extracellular release of DNA nets (traps) by leukocytes has been described as an important mechanism of the innate immune response in different infectious diseases including fungal infections. Purified human eosinophils release extracellular DNA traps (EETs) by different stimuli including bacteria and cytokines. *Aspergillus fumigatus* (AF) is an opportunistic filamentous fungus that may cause invasive aspergillosis, a pathological condition of high morbidity and mortality in immunocompromised patients. *In vivo*, eosinophils are recruited to the lung after exposure to AF and release cationic proteins, which demonstrate an important role in the elimination of this pathogen. In vitro, eosinophils present potent fungicidal activity against AF. However, the mechanisms that lead to recognition as well as the death of AF by eosinophils remain unknown. In this work we investigated whether eosinophils release EETs in response to AF and the mechanisms involved.

Methods: We isolated eosinophils from blood of healthy donors by negative immunomagnetic selection. Cells were then stimulated with AF in the ratios (fungus:cell) 1:1, 10:1, 50:1 and 100:1 being the release of EETs evaluated at different incubation times by a quantitative fluorimetric method and by confocal fluorescence microscopy.

Results: We observed that EETs were significantly released after 6, 9 and 12 h of incubation. The incubation time of 6h and the ratio (fungus:cell) 10:1 were then selected for further studies (control=84.08±10.71 FU, AF=281.6±33.82 FU, n=8, student's t test $p<0.05$, FU=fluorescence unit). Pretreatment of eosinophils for 30 min with DPI (20µmol/mL) (AF=307.2±50.21 FU, DPI=232.3±45.95 FU, n=7) or apocynin (100µmol/mL) (AF=248.50±38.47 FU, Apo=229.0±40.57 FU n=4), both inhibitors of reactive oxygen species (ROS), did not inhibit the AF-induced EETs release. However, the pretreatment with piceatannol (40µmol/mL) (AF=214.5±32.47 FU, PCT=97.08±13.12 FU, n=6, student's t test $p<0.05$) or OXSI (2µmol/mL) (AF=303.5±59.26 FU, OXSI=146.9±25.34 FU, n=6, student's t test $p<0.05$), both inhibitors of Syk tyrosine kinases, significantly inhibited the AF-induced EETs release.

Conclusion: Our results indicate that human eosinophils release EETs in response to AF through a mechanism which involves the Syk tyrosine kinases pathway, but independent of ROS.

Financial support: FAPERJ, CNPq and Capes

POSTER 11

CANNABINOID RECEPTOR 2 AUGMENTS HUMAN EOSINOPHIL RESPONSIVENESS VIA G GAQ/MEK/ROCK SIGNALING

Robert B. Frei, MSc[1], Miriam Peinhaupt, MSc[1], Gerald P. Parzmair, MSc[1], Silke Schranz, MSc[1], Akos Heinemann, MD[1], Eva. M. Sturm, PhD[1]

[1] Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

Background: Accumulation of activated eosinophils in tissue is a hallmark of allergic inflammation. The endocannabinoid 2-arachidonoylglycerol (2-AG) has been proposed to elicit eosinophil migration in a CB2 receptor/Gi/o-dependent manner. However, it has been claimed recently that this process also may involve other mechanisms such as cytokine priming, rapid 2-AG hydrolysis into eicosanoids, and the effect of 15-lipoxygenase metabolites.

Objective: Here we explored the direct contribution of specific CB2 receptor activation to human and murine eosinophil effector function.

Methods: Eosinophils were isolated from human peripheral blood. CB2 signaling was investigated in various functional in vitro assays. CB2 expression was confirmed by flow cytometry.

Results: The selective CB2 receptor agonist JWH-133 induced a moderate migratory response in eosinophils and furthermore enhanced chemoattractant-induced eosinophil activation. The receptor specificity of the observed effects was confirmed using the selective CB2 antagonist SR144528. Moreover, selective CB2 stimulation evoked a transient increase in intracellular Ca^{2+} and medi-

ated the activation of MAPK-kinase 1/2 (MEK 1/2) and Rho-associated protein kinase (ROCK) via a pertussis toxin (PTX)-insensitive G-protein.

Conclusion: These data emphasize the important immunomodulatory role of CB2 receptor ligands and provide new insights into the molecular mechanisms underlying the CB2-mediated priming of eosinophils.

Declaration of funding: E.S. received funding from the Franz Lanyar Stiftung of the Medical University of Graz (Grant Nr. 344) and by the Jubilaeumsfonds of the Austrian National Bank (Grant Nr. 14446). R.F. and M.P. were supported by the Austrian Science Fund FWF (DK-MOLIN W1241) and A.H. from the Austrian Science Fund FWF (Grant Nr. P22521) and Jubilaeumsfonds of the Austrian National Bank (Grant Nr. 14263).

POSTER 12

EOSINOPHIL ASSOCIATED CD48: REGULATION AND THE ROLE OF ITS SOLUBLE FORM IN STAPHYLOCOCCUS AUREUS ENTEROTOXIN B INDUCED EOSINOPHIL ACTIVATION

Roopesh S. Gangwar[1] and Francesca Levi-Schaffer[1]

[1]Pharmacology & Experimental Therapeutics Unit, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Background: Eosinophils, the main effector cells of late and chronic stages of allergic inflammation, express high levels of CD48, a glycosylphosphatidylinositol (GPI)-anchored cell-surface protein (mCD48) that also exists in a soluble form (sCD48). Increased levels of sCD48 are detected in plasma/serum of asthmatic patients. The study was undertaken to understand the regulation and expression of CD48 on eosinophils, mechanism of formation of sCD48 and its role in Staphylococcal enterotoxin B (SEB) induced eosinophilic inflammation.

Methods: Eosinophils were purified from human peripheral blood of mildly atopic volunteers. CD48 expression on eosinophils' membrane and intracellularly were studied by immunofluorescence. The cells were activated by SEB for up to 18 h and sCD48 in supernatants (ELISA) and mCD48 on cells (FC) were measured at different time points. Cell activation was monitored by CD11b expression. Eosinophils were pre-incubated either with phospholipases (PL) C or D, inhibitors or cycloheximide or Brefeldin A and then activated with SEB after which mCD48 and sCD48 were measured. SEB was preincubated with sCD48, then it was added to eosinophils and cell activation was determined by released EPO and IL-8 (ELISA), as well as by their chemotaxis (transmigration assay). Peritonitis was induced in mice by SEB injection (i.p.). After 48 h mice were euthanized and spleen, serum and peritoneal lavages were collected and analyzed (weight, FC, ELISA).

Results: Eosinophils contain an intracellular pool of CD48 mostly in the cytoplasm that partly colocalizes with granule proteins. SEB activated eosinophils released significantly ($p<0.05$) more sCD48 than the control from 3 h to up to 18 h. Elevated levels of sCD48 were found to be directly correlated ($p=0.0001$) to upregulation in CD11b expression on eosinophils. However, no correlation was observed between sCD48 and mCD48, the latter being nearly constant. Cell-associated PLC and PLD were found to be involved ($p<0.05$) in the cleavage of mCD48 from eosinophils to form sCD48. Blocking *de-novo* protein synthesis and transport in eosinophils decreased both mCD48 and sCD48 levels ($p<0.05$ and <0.01 respectively) indicating that the intracellular CD48 is transported to the membrane to maintain mCD48. Interestingly, SEB bound to sCD48, as eosinophils were found to be significantly less activated by SEB preincubated with sCD48 [lower EPO ($p<0.05$), IL-8 ($p<0.01$) release and transmigration ($p<0.01$)]. In a mouse model of SEB induced peritonitis sCD48 was significantly increased ($p<0.01$) in the peritoneal lavage and directly correlated to eosinophil numbers ($p=0.0017$). Moreover, pretreatment with sCD48 (i.p. 30 min before SEB injection) significantly downregulated the peritonitis as detected by decreased total infiltrating cells, eosinophils, cell activation and other inflammatory parameters.

Conclusions: This work describes the CD48 expression on eosinophils and provides the mechanism of formation of sCD48. Moreover it offers further insight into understanding the cleavage of other GPI-anchored molecules from the cell surface. The results also show sCD48's role as a functional antagonist in SEB induced eosinophil activation *in vitro* and in a mouse model of peritonitis, thus highlighting the importance of sCD48 as a potential drug candidate in eosinophilic inflammation.

Grant support: This work was supported by MAARS EU 7th framework (grant no. HEALTH-F2-2011-261366), Hebrew University of Jerusalem (Israel)-Drexel University (Philadelphia, PA, USA) Joint grant (no. 0304917) and Israel Science Foundation (grant 213/05), Aimwell Charitable Trust (London, UK). Roopesh Singh Gangwar acknowledges the financial support of "PBC postdoctoral fellowship for Indian and Chinese students" from The Hebrew University of Jerusalem, Israel.

POSTER 13

ADIPOSE TISSUE OF CCR2 DEFICIENT MICE DISPLAY INCREASED EOSINOPHIL ACCUMULATION, TYPE 2 CYTOKINE EXPRESSION, AND ALTERNATIVE MACROPHAGE POLARIZATION.

W. Reid Bolus[*†], Dario A. Gutierrez[*†2], Arion J. Kennedy[*], Emily K. Anderson-Baucum[*3], and Alyssa H. Hasty[*‡]

Adipose tissue (AT) inflammation during obesity is mediated by immune cells and closely correlates with systemic insulin resistance. In lean AT, eosinophils are present in low but significant numbers and capable of promoting macrophage alternative activation in an IL-4/IL-13 dependent manner. In wild type (WT) mice, obesity causes the proportion of AT eosinophils to decline, concomitant with inflammation and classical activation of AT macrophages. In this study we show that CCR2 deficiency leads to increased eosinophil accumulation in AT. Furthermore, in contrast to WT mice, the increase in eosinophils in $CCR2^{-/-}$ AT is sustained and even amplified during obesity. Interestingly, a significant portion of eosinophils are found in crown-like structures in AT of obese $CCR2^{-/-}$ mice, which is the first time eosinophils have been shown to localize to these inflammatory hot spots. $CCR2^{-/-}$ bone marrow precursors displayed increased expression of various key eosinophil genes during *in vitro* differentiation to eosinophils, suggesting a potentially altered eosinophil phenotype in the absence of CCR2. In addition, the proportion of eosinophils in AT positively correlated with local expression of *Il5*, a potent eosinophil stimulator. The increase in eosinophils in $CCR2^{-/-}$ mice was detected in all white fat pads analyzed and in the peritoneal cavity, but not in bone marrow, blood, spleen, or liver. In AT of $CCR2^{-/-}$ mice, increased eosinophil number positively correlated with M2-like macrophages, expression of the Treg marker *Foxp3,* and type-2 cytokines, *Il4, Il5,* and *Il13*. This is the first study to link CCR2 function and regulation of AT eosinophil accumulation.

† Authors contributed equally

‡Corresponding Author

*Vanderbilt University School of Medicine, Department of Molecular Physiology and Biophysics, Nashville TN, 37232, USA

‡ VA Tennessee Valley Healthcare System, Nashville TN, 37212, USA

1. This project was supported by a Veterans Health Administration Merit Award (5I01BX002195) and by an American Heart Association Established Investigator Award (12EIA8270000) to AHH. DAG was supported by an individual NRSA (DK091128), WRB was supported by the Molecular Endocrinology Training Grant (DK07563), and AJK was supported by a postdoctoral fellowship from the ADA. EKAB was supported by a pre-doctoral American Heart Association fellowship (12PRE11910047). AHH was also supported by NIH grant HL089466 and an American Diabetes Association Career Development Award (1-07-CD-10). Flow Cytometry and confocal imaging experiments were performed with scholarship provided by the Vanderbilt Digestive Disease Research Center (DK058404).

2. Current Address: German Cancer Research Center, Department of Cellular Immunology, Heidelberg Germany, D-69120.

3. Current Address: Department of Medicine, Indiana University School of Medicine.

POSTER 14

PROLONGED RSK AND RPS6 PHOSPHORYLATIONS BY IL-3 INCREASES TRANSLATION IN HUMAN EOSINOPHILS

Stephane Esnault[1], Elizabeth A. Kelly[1], Mats W. Johansson[2], Zhong-Jian Shen[3], James S. Malter[3], Nizar N. Jarjour[1]

[1]Department of Medicine, Allergy, Pulmonary, and Critical Care Medicine Division, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA [2]Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, [3]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA.

Background: IL-5 is a major therapeutic target to reduce eosinophilia. However, the eosinophil-activating cytokines IL-5, IL-3, and GM-CSF are all present in asthma. Due to the functional redundancy of these 3 cytokines on eosinophils and the loss of IL-5 receptor on airway eosinophils, it is important to take IL-3 and GM-CSF into account to efficiently reduce tissue eosinophil functions. Moreover, these 3 cytokines signal through a common ß-chain receptor, and yet differentially affect protein production in eosinophils. Notably, the increased ability of IL-3 to induce semaphorin-7A protein production without affecting mRNA level suggests a unique influence by IL-3 on translation. The purpose of this study is to identify the mechanisms by which IL-3 distinctively affects eosinophil function compared to IL-5 and GM-CSF, with a focus on protein translation.

Methods: Peripheral blood eosinophils isolated from mild asthmatic subjects were used to study intracellular signaling and protein translation in cells activated with IL-3, GM-CSF or IL-5. Airway eosinophils were obtained 48 h after a segmental allergen challenge to analyze *in vivo* intracellular signaling.

Results: We establish that, unlike GM-CSF or IL-5, IL-3 triggers prolonged signaling through activation of RPS6 and the upstream kinase, p90S6K (RSK). Blockade of RSK activation inhibited phosphorylation of RPS6 and IL-3-enhanced semaphorin-7A translation.

Furthermore, in an allergen-challenged environment, *in vivo* phosphorylation of RPS6 and RSK was enhanced in human airway compared to circulating eosinophils.

Conclusion: Our findings provide new insights into the mechanisms underlying differential activation of eosinophils by IL-3, GM-CSF, and IL-5. These observations place IL-3 and its downstream intracellular signals as novel molecular targets that should be considered to modulate eosinophil functions. By enhancing RSK and RPS6 activity, IL-3 likely increases many proteins. Their identification is critical to appreciate the potential influence and value that blocking this pathway would have on eosinophil functions.

This work was supported in part by a Program Project Grant (NIH HL088594) and the University of Wisconsin Institute for Clinical and Translational Research (NCRR/NIH 1UL1RR025011).

POSTER 15

ALTERATIONS IN THE POLARIZED MORPHOLOGY OF INTERLEUKIN-5-STIMULATED EOSINOPHILS UPON ADHESION TO PERIOSTIN, AND PERIOSTIN CLEARANCE INVOLVING ADAM8

Mats W. Johansson[1], Manav Khanna[1], Emily M. Wilkerson[2], Joshua J. Coon[1,2], and Deane F. Mosher[1,3]

Departments of [1]Biomolecular Chemistry, [2]Chemistry, and [3]Medicine, University of Wisconsin, Madison, WI, USA

Background: Interleukin-5 (IL-5) causes eosinophils in suspension to polarize with cortical filamentous actin (F-actin) and granules moving to one pole and the nucleus to the opposite pole into a specialized uropod, the nucleopod, which is enriched in receptors including P-selectin glycoprotein ligand-1 (PSGL-1). IL-5-stimulated eosinophils adhere to and migrate on periostin, an extracellular matrix (ECM) protein upregulated by T helper cell type 2 (Th2) mediators in the asthmatic airway. We asked if and how the polarized morphology evolves when IL-5-stimulated eosinophils adhere to periostin.

Methods: Purified human blood eosinophils adhering to adsorbed periostin in the presence or absence of IL-5 were imaged at different time points by fluorescent microscopy, including confocal microscopy.

Results: After adhesion for 10 min in the presence of IL-5, eosinophils were polarized with PSGL-1 over the nucleopod and F-actin distributed at the opposite end. After 60 min with IL-5, eosinophils were more spread, the nucleus was more centralized, PSGL-1 was localized in a crescent at one side of the nucleus, and F-actin was localized on the other side in podosomes, punctate adhesive contacts that are distinct from classical focal adhesions. The podosomes also contained gelsolin and actin-related protein (Arp) 3. In the absence of IL-5, adherent eosinophils were rarer, less spread, and did not have podosomes. The periostin layer was cleared progressively around adherent eosinophils. Clearance was attenuated by metalloproteinase inhibitors. Label-free quantitative mass spectrometry detected disintegrins and metalloproteinases (ADAMs) and matrix metalloproteinases (MMPs) in eosinophils in the following order of abundance: ADAM8, MMP25, ADAM10, ADAM19, MMP9, ADAM17, MMP8, and ADAM28. A novel splice variant of ADAM8 lacking the transmembrane domain was also detected. The five most abundant metalloproteinases were detected by immunofluorescence but did not localize to podosomes. Peroistin clearance was attenuated by inclusion of anti-ADAM8 antibody in the medium.

Conclusions: Upon adhering to periostin in the presence of IL-5, eosinophils transition from a polarized morphology similar to that in suspension to a more spread morphology with podosomes. Periostin is lost from the substrate in the vicinity of eosinophils in a process that involves ADAM8, possibly in a soluble form. The morphology of polarized suspended eosinophils, therefore, appears to facilitate acquisition of a migratory cellular phenotype upon adhesion and the ability to remodel periostin-rich ECM in a manner involving ADAM8. Eosinophil ADAM8-mediated remodeling of periostin-containing matrix may be a significant process in the asthmatic airway.

Grant support: This work was supported in part by a Program Project Grant on the Role of Eosinophils in Airway Inflammation and Remodeling P01 HL088584 (PI: Nizar N. Jarjour) from the NIH-NHLBI and a seed grant from the University of Wisconsin Carbone Cancer Center to DFM and JJC. EMW gratefully acknowledges support from NIH-funded Research Training in Hematology T32 HL007899.

POSTER 16

COMPARATIVE PROTEOMICS OF UNACTIVATED AND ACTIVATED PERIPHERAL BLOOD EOSINOPHILS

Emily M. Wilkerson[1], Mats W. Johansson[2], Alexander S. Hebert[4], Michael S. Westphall[4], Deane F. Mosher[2,3], and Joshua J. Coon[1,2,4]

Departments of [1]Chemistry, [2]Biomolecular Chemistry, and [3]Medicine, University of Wisconsin, Madison, WI, USA; [4]Genome Center of Wisconsin, University of Wisconsin, Madison, WI, USA

Background: Upon activation, blood eosinophils undergo shape change and relocalization of surface receptors. A comprehensive knowledge of the eosinophil proteome, which to date has been limited to only a few hundred proteins, is required to understand such activation.

Methods: For global analysis of unactivated eosinophils, blood eosinophils purified from three individuals were combined, lysed by probe sonication, digested with trypsin, and desalted. The sample was enriched for phosphorylation using immobilized metal-affinity chromatography (IMAC). The enriched and non-enriched samples were pre-fractionated using high pH reversed-phase chromatography. The fractions were analyzed on a nanoLC coupled to an Orbitrap Fusion (Thermo Scientific). MaxQuant was used to search, filter to 1% false discovery rate, and provide label free quantitation. Phosphorylation sites were localized with the PhosphoRS algorithm. For the comparison between activated and unactivated cells, eosinophils were purified from five different volunteers. After purification, each preparation was split in half, one half was incubated with interleukin-5 (IL-5) for 5 minutes and the other was left unperturbed. The ten samples were individually lysed, digested, desalted, and tagged using 10-plex Tandem Mass Tags (TMT, Thermo Scientific). The tagged samples were recombined in equal amounts and analyzed as described above except the resolution was increased for the MS2 scan to allow quantification of the reporter ions from the fragmented mass tags. The software suite COMPASS (Coon OMSSA Proteomic Analysis Software Suite) was used to analyze the data and supplemented with localization of the phosphorylation data using PhosphoRS.

Results: We identified over 100,000 unique peptides that mapped to 6,899 unique proteins. From these data, we estimate the abundance rank order of 6,855 of these proteins through intensity-based absolute quantification (iBAQ). Five of the 11 most abundant proteins are stored in the granules that are characteristic of eosinophils. From the IMAC-enriched samples, 5,336 sites of phosphorylation were localized. In the isobaric labeling experiment to compare the proteomes and phosphoproteomes of unactivated and activated eosinophils, were able to quantify 4,447 proteins and 2,068 sites of phosphorylation. Acute activation with IL-5 resulted in a 2-fold change in the abundance of only five of the proteins, whereas 150 of the phosphosites were significantly changed between the two conditions ($P<0.05$, t test with Bonferroni correction).

Conclusions: These protein and phosphorylation data provide unparalleled coverage of the eosinophil proteome. We are currently in the process of validating and interpreting the phosphorylation data as well as assessing significance of changes across multiple pathways. Variation among individuals will also be explored further using the current data set. These experiments should provide a solid basis for using comparative proteomics to study eosinophil activation and variability in individual responses.

Grant support: This work was supported in part by a Program Project Grant on the Role of Eosinophils in Airway Inflammation and Remodeling P01 HL088584 (PI: Nizar N. Jarjour) from the NIH-NHLBI and a seed grant from the University of Wisconsin Carbone Cancer Center to DFM and JJC. EMW gratefully acknowledges support from NIH-funded Research Training in Hematology T32 HL007899.

POSTER 17

GALECTIN-1: ROLE IN REGULATION OF EOSINOPHILIA AND ALLERGIC AIRWAY INFLAMMATION

Xiao Na Ge[1], Sung Gil Ha[1], Idil Bastan[1], Yana G. Greenberg[1], Amrita Rao[1], Savita P. Rao[1], Gabriel A. Rabinovich[2] and P. Sriramarao[1]

[1]Dept. of Veterinary & Biomedical Sciences, University of Minnesota, St. Paul, Minnesota and [2]Laboratory of Immunopathology, Institute of Biology and Experimental Medicine (IBYME, CONICET), C1428, Buenos Aires, Argentina

Background: Galectin-1 (Gal-1) is a glycan-binding protein that regulates various aspects of inflammation by virtue of its ability to act both extracellularly as well as intracellularly and affect processes such as cell adhesion, migration, activation, signaling, proliferation, differentiation and apoptosis. While multiple in vivo studies have indicated an anti-inflammatory/pro-resolution role for Gal-1, the role of this molecule in regulating allergic airway inflammation or eosinophil function is not known.

Methods: Expression of Gal-1 by bone marrow-derived murine eosinophils and its effect on function of these cells was examined in vitro using flow cytometry and confocal microscopy along with apoptosis, adhesion and migration assays. The role of Gal-1 in vivo, was examined in a mouse model of acute allergen (ovalbumin)-induced airway inflammation in wild type (WT) versus Gal-

1-deficient (*Lgals1-/-*) mice. Gal-1 expression, lung cellular inflammation, cytokines and chemokines, airway hyperreactivity and airway mucus secretion was assessed in these mice.

Results: Allergen exposure significantly induced expression of Gal-1 in the lungs of WT mice relative to control mice ($p < 0.04$) attributable to the recruitment of Gal-1-expressing inflammatory cells, including eosinophils, as well as increased Gal-1 in extracellular spaces. In vitro, extracellular Gal-1 exerted varying effects on eosinophils that were glycan-dependent and inhibited by lactose. At lower concentrations (0.1 - 0.25 μM), Gal-1 induced eosinophil adhesion to VCAM-1 but inhibited eotaxin-1-induced migration in a dose-dependent manner ($p < 0.02$ versus untreated cells) and was associated with inhibition of ERK activation. Exposure to higher concentrations of Gal-1 (1-5 μM) resulted in rapid caspase-independent apoptosis and disruption of the F-actin cytoskeleton. The increased eosinophil adhesion when exposed to low concentrations of Gal-1 was not associated with increased α4 (CD49), β2 (CD18), αL (CD11a), αM (CD11b), L-selectin or CCR3 expression. However, decreased α4 and CCR3 was noted in eosinophils treated with Gal-1 at higher pro-apoptotic concentrations. Allergen-challenged Gal-1-deficient (*Lgals1-/-*) mice exhibited significantly increased recruitment of eosinophils and $CD3^+$ lymphocytes in the airways ($p<0.02$) as well as elevated peripheral blood and bone marrow eosinophils ($p<0.03$) relative to allergen-challenged WT mice. These mice had an increased propensity to develop airway hyperresponsiveness exhibiting significantly elevated airway reactivity to inhaled methacholine even at lower doses relative to WT counterparts ($p<0.05$). In addition, allergen-challenged Gal-1 deficient mice displayed significantly elevated levels of TNF-α in the lung tissue relative to allergen-challenged WT mice ($p<0.05$) while levels of IL-5 and IL-13 also tended to be higher.

Conclusions: Our in vitro and in vivo findings together suggest that Gal-1 is induced in the lungs in response to allergen challenge and plays an important role in potentially suppressing/resolving airway inflammation by controlling/limiting eosinophil recruitment to allergic airways and promoting apoptosis.

POSTER 18

SPECT/CT TO QUANTIFY EOSINOPHIL MIGRATION INTO THE LUNGS

Chrystalla Loutsios[1], Neda Farahi[1], Rosalind Simmonds[1], Daniel Gillett[2], Ian Cullum[2], Chandra Solanki[2], Sarah Heard[2], Laurence S.C. Lok[2], John Buscombe[2], Alison M. Condliffe[1], A. Mike Peters[3], Edwin R. Chilvers[1]

[1]Division of Respiratory Medicine, Department of Medicine, University of Cambridge, Cambridge, UK, [2]Department of Nuclear Medicine, Addenbrooke's Hospital, Cambridge, UK, [3] Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, Brighton, UK.

Background: Eosinophils are key mediators of allergic inflammation. The ability to localise and quantify eosinophilic inflammation is important both clinically and to test the efficacy of novel therapeutics. Existing biomarkers are indirect or invasive so there remains a need for a less invasive method to localise eosinophilic inflammation in the lungs.

Methods: Granulocytes were isolated from autologous peripheral blood by plasma-Percoll density gradient separation. Eosinophils were then isolated by negative selection using anti-CD16 magnetic microbeads, labelled with ^{99m}Tc-HMPAO, and injected into 9 healthy subjects, 10 asthmatics and 3 patients with focal eosinophilic lung inflammation. Initial cell distribution was monitored by dynamic imaging over the chest for 40 min post-injection. Frequent serial peripheral blood samples were taken and lung activity determined from 3 sequential SPECT images obtained up to 9 h post-injection. Activity was anatomically co-registered from a single low-dose CT scan. The rate of eosinophil clearance into the lungs was measured by Patlak analysis, using peripheral blood for input function, and multiplied by the blood eosinophil count to give the rate of eosinophil migration into the lungs.

Results: Labelled cells displayed rapid first-pass transit through the lungs before localising in the liver, spleen and bone marrow. Lung eosinophil migration was 194 ± 66 cells/min/ml in asthmatics ($p<0.1$) and 6051 ± 4593 in patients with focal pulmonary inflammation ($p<0.01$), compared with 46 ± 22 in healthy subjects.

Conclusion: Autologous radiolabelled eosinophils coupled to SPECT/CT are able to localise pulmonary eosinophilic inflammation in humans and therefore have the potential for testing novel therapeutics.

Grant support: This work was supported by Asthma-UK [08/11], the Medical Research Council [grant number MR/J00345X/1], the Wellcome Trust [grant number 098351/Z/12/Z], and Cambridge NIHR Biomedical Research Centre.

POSTER 19

A NOVEL PROTEASE, PRSS33 (SERINE PROTEASE 33; EOS), IS SPECIFICALLY AND CONSTITUTIVELY EXPRESSED IN HUMAN EOSINOPHILS

Sumika Toyama[1,2], Akio Matsuda[1], Hajime Karasuyama[2], Hirohisa Saito[1], Susumu Nakae[3], Kenji Matsumoto[1]

[1] Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Department of Immunology, [2] Department of Immune Regulation, Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, [3] Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Background: PRSS33 (serine protease 33; EOS) is a novel serine protease, first identified in activated human macrophages in 2003. PRSS33 is evolutionarily conserved throughout all mammals, suggesting that it plays some critical role in the survival of all mammals. However, its physiological/pathological function is completely unknown to date. This study investigated PRSS33 expression and function in human eosinophils.

Methods: Human eosinophils and other cell types were purified from peripheral blood of healthy or mildly allergic donors by density gradient sedimentation and negative/positive selections using immunomagnetic beads. Expression of PRSS33 by eosinophils and also other blood cells and human hematopoietic stem cell-derived mast cells was determined by microarray analysis, qPCR and immunofluorescent staining. In addition, eosinophils were stimulated with 10 ng/ml GM-CSF or 0.1 ng/ml IL-5, and then the PRSS33 concentration in culture supernatants and cell lysates as well as cell surface expression of PRSS33 were determined by ELISA and flow cytometry, respectively.

Results: Human eosinophils constitutively expressed PRSS33 mRNA, and exposure to GM-CSF or IL-5 did not affect the mRNA expression levels. Microarray analyses demonstrated that among all blood cells and mast cells, only eosinophils expressed PRSS33. Immunohistochemical staining showed that PRSS33 was co-localized with an eosinophil granule protein (ECP). PRSS33 was not detected in the culture supernatant of eosinophils even after stimulation with GM-CSF or IL-5.

Conclusions: PRSS33 is expressed constitutively and specifically by human eosinophils. Based on this protein's amino acid sequence and the finding that it was not released even after degranulation, PRSS33 is likely a membrane-bound molecule. PRSS33 has two conserved domains that show high homology to human tryptase-gamma, which reportedly played critical roles in an experimental chronic obstructive pulmonary disease and colitis. Further studies are needed to clarify the function of PRSS33 in eosinophils.

This study was supported by NiBio (#10-43 to KM) and JSPS KAKENHI (#15K09560 to KM) grants.

POSTER 20

LEUKOTRIENE B4: UNDERAPPRECIATED REGULATOR OF HUMAN EOSINOPHILS

Julie S. Lefebvre, Anne-Sophie Archambault, Marie-Chantal Larose, Michel Laviolette, and Nicolas Flamand.

Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Faculté de médecine, Université Laval, Québec, QC G1V 4G5, Canada.

Background: Elevated blood and sputum eosinophil counts correlate with asthma severity and are utilized in clinic as readouts for treatment efficacy. However, some severe asthmatics remain symptomatic despite high doses corticosteroids and still present persistent elevated blood and sputum eosinophil counts. It is thus imperative to understand how eosinophils migrate to the tissue in order to develop alternative therapeutic strategies that would block the recruitment and activation of eosinophils in the lungs of asthmatics. LTB4 is a potent chemoattractant and activator of murine eosinophils. In contrast, some groups published that LTB4 did not activate human eosinophils while others documented the opposite. The most recent consensus is that LTB4 does not regulate human eosinophils. The aim of this study was to revisit the involvement of LTB4 at regulating human eosinophil responses.

Methods: Using freshly purified human eosinophils from healthy and asthmatic patients we evaluated the impact of LTB4 on human eosinophil functions. BLT1 expression was determined by FACS. The chemotactic response of eosinophils was assessed using modified Boyden-chamber assays. Reactive oxygen species (ROS) production was measured using the absorbance of reduced cytochrome c.

Results: We confirmed that human eosinophils express the LTB4 receptor 1, with comparable levels to those observed in human monocytes. In addition, LTB4 is in fact a good chemotactic agent for human eosinophils with maximal migration obtained between 10 and 100 nM after 2h. We did not observed significant migration rate when we compared eosinophils from healthy volunteers, mild asthmatics or severe eosinophilic asthmatics. Moreover, although the chemotactic response of eosinophils to LTB4 did not change according to asthma severity, we found that eosinophils from asthmatics (mild or severe) produce more ROS in response

to LTB4 than those from healthy donors. This increased in ROS production was not observed in neutrophils isolated from the same donors. Finally, we found that the increased ROS production induced by LTB4 in asthma correlated with a decrease ROS production induced by fMLP. there was an inverse correlation between the fMLP-induced and the LTB4-induced ROS production

Conclusions: Altogether, our data show that LTB4 recruits and activates eosinophils, and therefore likely contributes to eosinophilic inflammation in humans, notably in asthma.

Grant support: supported by grants the Canadian Institutes of Health Research (NF), the Fonds de recherche du Québec-Santé (NF) and its Respiratory Health Network (MCL, NF, ML, CL), and the Natural Sciences and Engineering Research Council of Canada (NF).

POSTER 21

METABOLISM OF EXOGENOUS ARACHIDONOYL-ETHANOLAMIDE AND 2ARACHIDONOYL-GLYCEROL BY HUMAN EOSINOPHILS.

Anne-Sophie Archambault, Marie-Chantal Larose, Caroline Turcotte, Julie S Lefebvre, Véronique Provost, Cyril Martin, Michel Laviolette, and Nicolas Flamand.

Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Faculté de médecine, Université Laval, Québec, QC G1V 4G5, Canada.

Background: Eosinophils are leukocytes involved in numerous inflammatory diseases such as asthma and possibly obesity. How they migrate to the tissues is not completely defined but it involves chemokines and/or bioactive lipids, noteworthy prostaglandin (PG) D2 and 5-oxo-eicosatetraenoate (5-KETE), and possibly 2arachidonoyl-glycerol (2-AG). In this respect, we documented that the combination of IL-5 and 2-AG induces an eosinophil migration comparable to that of CCL11, a chemokine inducing the selective recruitment of eosinophils. Noteworthy, the 2-AG-induced eosinophil migration was prevented by 15-lipoxygenase (LO) inhibitors, suggesting an involvement of the 15-LO pathway in the regulation of eosinophil functions by endocannabinoids. We thus postulated that 2-AG and arachidonyl-ethanolamide (AEA) were metabolized by the 15-LO.

Results: We developed and analytical HPLC method that separates numerous AEA-, 2-AG-, and arachidonic acid (AA)-derived 15-LO metabolites as well as cysteinyl-leukotrienes (LT). AEA was mainly metabolized by eosinophils into 15-HETE-EA. This metabolism of AEA was concentration- and time-dependent. Moreover, it not modulated by platelet-activating factor (PAF), which activates the 5-LO pathway. In absence of PAF, human eosinophils mainly metabolized 2-AG into 15-HETE. In presence of PAF, human eosinophils mainly metabolized 2-AG into 15-HETE, and LTC4. Another set of experiments was also performed in which eosinophils were incubated in presence of the 2AG hydrolysis inhibitors MAFP or JZL184. Under these conditions, 2-AG was mainly metabolized into a mixture of 15-HETE-*sn2*-glycerol and 15-HETE-*sn1*-glycerol. PAF did not modulate the levels of the glyceryl metabolites of 15-HETE we observed.

Conclusions: AEA and 2-AG are metabolized by human eosinophils. AEA is mainly metabolized by the 15-LO pathway. 2-AG can be hydrolyzed into AA and eicosanoids from the 5- and the 15-LO pathways. When 2-AG hydrolysis is prevented, 2-AG is mainly metabolized by the 15-LO pathway. This study underscores the importance to characterize the biological functions of endocannabinoid-derived 15-LO metabolites in the regulation of inflammation in order to define the impact of systemic MAG lipase inhibition in the regulation of inflammation.

Funding: this work was supported by grants from the Canadian Consortium for the Investigation of Cannabinoids (CT), the Canadian Institutes of Health Research (NF), the Fonds de recherche du Québec-Santé (NF) and its Respiratory Health Network (MCL, NF, ML, CL), and the Natural Sciences and Engineering Research Council of Canada (NF).

POSTER 22

EOSINOPHILS DRIVE CARDIAC REMODELING AND DEVELOPMENT OF HEART FAILURE

Nicola L. Diny[1,2], Jobert G. Barin[1], Monica V. Talor[1], Noel R. Rose[1,2], Daniela Čiháková[1,2]

[1]Department of Pathology, the Johns Hopkins University School of Medicine and [2]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA,

Eosinophilic myocarditis is a rare, potentially fatal disease. Disease etiology may be associated with increased eosinophilia due to idiopathic hypereosinophilic syndrome, hypersensitivity, or parasitic infestation. Frequently, the cause of the disease remains unknown. Cardiac disease is the major cause of morbidity and mortality in patients with hypereosinophilic syndrome. Beside eosinophilic infiltration, additional features of eosinophilic myocarditis include myocyte necrosis, fibrosis, thrombosis, and cardiac remodeling. To investigate the role of eosinophils in the pathogenesis of eosinophilic myocarditis, and their contribution to cardiac remodeling and heart failure, we developed a model of eosinophilic myocarditis by inducing experimental autoimmune myocarditis (EAM) in mice expressing IL-5 under the CD3 promoter (IL-5^{Tg}). EAM was induced with myocarditogenic peptide emulsified in

Complete Freund's Adjuvant injected twice on day 0 and 7 of EAM. On day 21 IL-5^{Tg} mice developed massive eosinophilic cardiac inflammation that resulted in severe inflammatory dilated cardiomyopathy (DCMi) and heart failure on day 45. Thus, eosinophils elicit development of DCMi and heart failure.

We have previously reported that mice deficient in both IFNγ and IL-17A develop severe EAM with a third of the cardiac infiltrate comprised by eosinophils. Crossing the eosinophil-deficient ΔGATA1 allele onto IFNγ$^{-/-}$/IL17A$^{-/-}$ mice protected these animals from heart failure and death during EAM. Cardiac infiltrates in WT mice with EAM are 1-3% eosinophils. These findings prompted us to examine whether even small numbers of eosinophils in cardiac infiltrate would be sufficient to negatively impact cardiac function. We found that eosinophil-deficient ΔGATA1 mice developed myocarditis similar to WT mice, but were completely protected from its sequelae, inflammatory dilated cardiomyopathy (DCMi) and heart failure. This indicated that eosinophils are crucial for progression of myocarditis to DCMi. Moreover, even relatively small numbers of eosinophils could drive cardiac remodeling and heart failure. This novel finding was further supported by the observation that naïve IL-5^{Tg} mice have mild, diffuse eosinophilic cardiac infiltrates without myocyte necrosis, yet demonstrated significantly compromised cardiac function, compared to WT animals. To discriminate between the effects of IL-5 and eosinophils in this model, we crossed IL-5^{Tg} mice to eosinophil-deficient ΔGATA1 mice. Resulting ΔGATA1IL-5^{Tg} mice had high serum levels of IL-5 but lacked eosinophils and had preserved cardiac function.

Recently, eosinophils have been shown to drive several physiological processes through their production of IL-4. We therefore assessed IL-4 producing cell types in myocarditis using IL-4 reporter mice and found eosinophils to be the major IL-4 expressing cell type in the EAM. Additionally, IL-4-deficient mice developed myocarditis but were completely protected from DCMi, matching the phenotype of ΔGATA1 mice. IL-4 treatment of adult mouse cardiac fibroblasts *in vitro* induced expression of CCL11 and stimulated secretion of pro-inflammatory cytokines and chemokines IL-1β, TNF-α and CCL2. In conclusion, eosinophils are required for progression of myocarditis to DCMi, drive severe DCMi when present in large numbers, and likely mediate this process through IL-4.

Supported by NIH/NHLBI R01HL118183 (DC) and R01HL113008 (NRR).

POSTER 23

A COMPARATIVE ANALYSIS OF EOSINOPHIL CELL SURFACE MARKERS IN EOSINOPHILIC DISORDERS

Fanny Legrand[1], Shakuntala Rampertaap[2], Sergio Rosenzweig[2], Joseph Monsale[2], Kimberly Romito[2], Nicole Holland-Thomas[3], Paneez Khoury[1], Amy D. Klion[1]

[1] Human Eosinophil Section Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, MD; [2] Department of Laboratory Medicine, Warren Magnusson Clinical Center, National Institutes of Health, Bethesda, MD, [3] Clinical Parasitology Section, Laboratory of Parasitic Diseases, Clinical Research Directorate/CMRP, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

Background: The number of peripheral blood eosinophils in an individual patient is a reflection of the balance between eosinophilopoiesis, apoptosis and eosinophil recruitment to tissues. Eosinophils express numerous surface receptors that are regulated *in vivo* and play a role in these processes. Consequently, surface phenotyping of blood eosinophils by flow cytometry is of potential interest as a means to better understand the factors that contribute to eosinophil-related pathogenesis in patients with eosinophilia. The aim of the present study was to characterize the expression of a set of surface markers associated with activation (CD69), apoptosis (Siglec-8) and migration (CD11a, VLA-4 and CCR3) on peripheral blood eosinophils from normal donors and patients with eosinophilic disorders of diverse etiologies.

Methods: Eosinophil surface receptor expression was quantified by flow cytometry using whole blood from 23 normal donors (ND) and 58 subjects with varied eosinophilic disorders (EOS). EOS subjects were classified by diagnosis and disease status (active, in remission on treatment, resolved) at the time of flow cytometric analysis. Absolute eosinophil count (AEC), symptoms and medications were also recorded. Siglec-8 expression was determined on blood eosinophils gated as CD45^{+}SSC^{Hi}VLA4^{+}; the other markers were quantified on CD45^{+}SSC^{Hi}Siglec-8^{+} cells. VLA4, CD11a, CCR3, and Siglec8 surface levels are presented as Geometric Mean Fluorescence Intensity (GM MFI) and CD69 as percentage of cells expressing the marker.

Results: Compared to eosinophils from ND, eosinophils from active EOS subjects expressed significantly less VLA4 (GM MFI 316 vs. 470, $P<0.001$) and CCR3 (GM MFI 1498 and 2343, $P<.001$). Furthermore, CCR3 was negatively correlated with AEC in the active EOS subjects ($r=-0.26, P<.05$) but not in ND. Although there was no significant difference in CD69 or Siglec-8 expression between the two groups, CD69 expression was positively correlated with AEC in the active EOS group ($r=0.28$, $P<.05$). Eight patients with hypereosinophilic syndrome (HES) were phenotyped before and after treatment with steroids. Of the 6 patients who experienced improvement in symptoms and eosinophilia post-treatment, CCR3 expression rose in 6/6 from a GM MFI of 1073 to 2017 ($p<0.05$,

Wilcoxon matched pairs test). Although CD69 expression decreased in 5 of the 6 steroid responders, the decrease was not statistically significant (GM 21% to 4%, *P*=0.12). No trend was seen for Siglec-8, VLA4 or CD11a expression.

Comparison of surface receptor expression on eosinophils in the active EOS subjects classified by diagnosis to ND was notable for significantly increased expression of CD69 in patients with myeloproliferative HES and increased expression of Siglec-8 in subjects with hypereosinophilia of unknown significance (HEUS; GM MFI 460 vs. 398 in ND, *P*=.01). Siglec-8 expression was not increased in EOS subjects in remission or resolved eosinophilia compared to ND.

Conclusions: These results demonstrate that regulation of surface markers on peripheral blood eosinophils occurs *in vivo* in eosinophilic subjects and suggest that specific patterns of expression may be associated with different etiologies and clinical manifestations of eosinophilic disease. Measurement of circulating levels of pro-eosinophilic cytokines/chemokines and multivariate analysis of surface marker expression is ongoing.

POSTER 24

THE AVAILABILITY AND UTILITY OF EOSINOPHIL-SPECIFIC REAGENTS AND MOUSE MODELS FROM LEE LABORATORIES

Nancy A. Lee and James (Jamie) J. Lee

Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, Arizona, USA

Background: One of the advantages of being "old" (think senior) investigators is that over the years we have built-up a large repository for reagents and mouse models as we pursued our studies of all things eosinophil. As was noted at recent IES meetings, our reagents, mice, and technical insights/advice are available to everyone; we are happy to get folks started and/or move their research forward.

Methods: To provide everyone a quick reference guide of the available reagents and mice from Lee Laboratories, including references related to their production, characterization, and/or use.

Results/Conclusions: As part of our ongoing studies and collaborative interactions, Nancy and I have strived to establish meaningful collaborations with other investigators that move eosinophil research forward and lead to interesting co-authored manuscripts and grants. However, we also wanted to make it possible for investigators who did not want to collaborate or who worked in areas where there was not common interest with Lee Laboratories to easily request our reagents/mice. In these cases, we simply ask that our reagents and/or mice are acknowledged in any manuscripts/papers, abstracts, or grants as having come from the "Laboratories of Drs. Nancy and Jamie Lee". The only restrictions Mayo Clinic have put on all of our activities (i.e., the transfer and distribution of materials/mice not subject to intellectual property licenses) are the assessment of small administrative fees to partially defray the costs to Mayo for the production, maintenance, and distribution of these materials. This system has worked with amazing efficiency over the years (at testament to Mayo Clinic infrastructure) and has allowed the transfer of reagents/mice to laboratories around the world with many of these materials becoming the "gold standard" reagents/mice used in basic research and now even patient-based studies. In fact, the current tallies from our records indicate that over the last 20 years we have collaborated and/or provided Lee Laboratories materials to nearly 1400 investigators ~70 colleagues/year) in 400 different institutions from 35 countries.

This work is supported by Mayo Foundation, numerous research grants from the NIH/NCI as well as grants from the American Heart Association, and the American Lung Association, and sponsored research proposals from Merck, Schering Plough, Genetics Institute/Wyeth/Pfizer.

POSTER 25

EOSINOPHIL TRAFFICKING TO THE HEART IN EOSINOPHILIC MYOCARDITS IS DEPENDENT ON CCR3

Nicola L. Diny[1,2], Jobert G. Barin[1], Monica V. Talor[1], Noel R. Rose[1,2], Daniela Čiháková[1,2]

[1]Department of Pathology, the Johns Hopkins University School of Medicine, and [2]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA

Eosinophilic myocarditis, cardiac inflammation associated with interstitial infiltration of eosinophils, can occur in the context of hypereosinophilic syndrome or without peripheral eosinophilia. 20-50% of patients with hypereosinophilic syndrome develop eosinophilic cardiac infiltration. The most severe form of eosinophilic myocarditis, necrotizing eosinophilic myocarditis, is characterized by extensive cardiomyocyte necrosis and carries a dire prognosis. A sequela to myocarditis, inflammatory dilated cardiomyopa-

thy (DCMi) is a major cause of heart failure. DCMi develops in up to 30% of myocarditis patients. It is not known how eosinophils traffic to the heart and what their role is in disease progression.

In wildtype (WT) BALB/c mice, experimental autoimmune myocarditis (EAM) is induced by immunization with cardiac myosin. EAM progresses to DCMi and mice develop heart failure. To address the role of eosinophils in myocarditis and DCMi, we immunized eosinophil-deficient ΔGATA1 mice. While ΔGATA1 mice developed myocarditis similar to WT mice, ΔGATA1 mice were completely protected from DCMi, indicating that eosinophils are critical for progression of myocarditis to DCMi. We previously showed that mice lacking both key T helper cell (Th)1 and Th17 cytokines IFNγ and IL-17A ($IFN\gamma^{-/-}IL\text{-}17A^{-/-}$) develop severe heart failure and rapidly fatal eosinophilic myocarditis upon immunization. When crossed to eosinophil-deficient ΔGATA1 mice, resulting ΔGATA1 $IFN\gamma^{-/-}IL\text{-}17A^{-/-}$ mice showed reduced mortality and reduced cardiac dilation, suggesting an important pathogenic role for eosinophils in myocarditis and DCMi.

$IFN\gamma^{-/-}IL\text{-}17A^{-/-}$ mice express high levels of eotaxins (*Ccl11* and *Ccl24*) in the heart during myocarditis. To test the hypothesis that eosinophils traffic to the heart in response to eotaxin signaling, we compared eosinophil levels in $IFN\gamma^{-/-}IL\text{-}17A^{-/-}$ and $IFN\gamma^{-/-}IL\text{-}17A^{-/-}CCR3^{-/-}$ mice. Indeed, $IFN\gamma^{-/-}IL\text{-}17A^{-/-}CCR3^{-/-}$ mice showed significantly lower eosinophil numbers in the heart during EAM. Expression of CCR3 was important for eosinophil trafficking to the heart only in the context of high cardiac eotaxin levels. ΔGATA1Ix$IFN\gamma^{-/-}IL\text{-}17A^{-/-}$ mice expressed much higher eotaxin levels than ΔGATA1 mice during EAM. When eosinophils from CCR3-competent or -deficient donors were adoptively transferred into these two recipients, CCR3-dependent trafficking was only evident in ΔGATA1x$IFN\gamma^{-/-}IL\text{-}17A^{-/-}$ recipients, but not in ΔGATA1 recipients. This effect was limited to the heart, as CCR3-competent eosinophils were found in higher frequencies than CCR3-deficient eosinophils in the gut and thymus of both recipients. Gut and thymus are organs known to require eotaxin-signaling for eosinophil recruitment.

In conclusion, eosinophil trafficking to the heart is CCR3-dependent in the context of high cardiac eotaxin-levels, which are found in mice with eosinophilic myocarditis. Given the pathogenic role of eosinophils in myocarditis, CCR3 blockade may be a useful therapeutic approach.

This work was supported by NIH/NHLBI grants R01HL118183 to Daniela Čiháková and R01HL113008 to Noel R Rose.

POSTER 26

NOTCH SIGNALING IS REQUIRED FOR THE PRIMING-INDUCED ENHANCED MIGRATION OF HUMAN EOSINOPHILS IN VITRO, AND RECRUITMENT OF MOUSE EOSINOPHILS INTO ALLERGIC LUNGS IN VIVO

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

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

Background: Notch signaling is an evolutionarily conserved pathway wherein Notch receptor-expressing cells receive and integrate exogenous and intrinsic cues to regulate cellular functions, including activation, proliferation, differentiation, and migration. In the canonical pathway, γ-secretase-dependent cleavage of activated (i.e. ligand-bound) Notch receptors releases an intracellular domain (NICD) that translocates to the nucleus to turn on the transcription of Notch-responsive genes. We previously reported mature human eosinophils express Notch receptors and ligands, and implicated autocrine Notch signaling in GM-CSF-induced eosinophil polarization and chemokinesis.

Objectives: In the present study our objectives were to determine if: 1) Signaling through plasma membrane-expressed Notch receptor 1 is required for the enhanced migration observed in human eosinophils following priming with IL-5 family cytokines *in vitro*; and 2) Targeted disruption of Notch signaling in mouse eosinophils prevents their accumulation within allergic airways *in vivo*.

Methods: *In vitro assays with human eosinophils.* Purified human blood eosinophils were primed for 18 hours in medium alone, or containing GM-CSF, IL-5, or IL-3, in the presence of global Notch inhibitors (i.e. γ-secretase inhibitors), neutralizing antibodies against Notch receptor 1, or vehicle or isotype controls. Following priming, eosinophils were overlaid onto confluent, cytokine-activated human umbilical vein endothelial cell monolayers growing on transwell inserts. Transmigrated eosinophils were collected and quantified from lower wells. *In vivo mouse model.* BALB/c wild-type or eosinophil deficient ΔGATA1-/- mice were sensitized with i.p. OVA plus alum adjuvant on days 0, 7, and 14, and challenged with aerosolized OVA on days 21, 22, and 23. Wild-type mice received i.p. treatments with γ-secretase inhibitors or vehicle control prior to each aerosolized OVA challenge. Eosinophil deficient mice received an i.v. infusion of syngeneic eosinophils pretreated with γ-secretase inhibitors or vehicle control prior to the final aerosolized OVA challenge. For both models, twenty-four hours after the final OVA challenge BAL fluid was collected and eosinophils quantified.

Results: Constitutive activation of Notch receptor 1 was detected in freshly isolated human blood eosinophils. Global inhibition of γ-secretase cleavage or neutralization of surface-expressed Notch receptor 1 was sufficient to inhibit eosinophil transendothelial

migration across bare filters, or across non-activated or activated endothelium. *In vivo*, systemic treatment or targeted pre-treatment of eosinophils with γ-secretase inhibitor impaired eosinophil accumulation within allergic lungs.

Conclusions: Our data identify Notch signaling as a cell intrinsic pathway critical to the priming-induced enhanced migration of human and mouse eosinophils. One implication of these data is that Notch signaling pathways might represent a novel therapeutic target to control eosinophil accumulation in allergic diseases. A second implication is the anticipation that unintended effects on eosinophil migration may be encountered in patients currently receiving γ-secretase inhibitor therapies in cancer clinical trials.

Grant support: Work was funded by the following grants to LAS: AAAAI Women in Allergy Junior Faculty Development Award; American Heart Association Grant-in-Aid; and NIH R01 HL095699.

POSTER 27

TUMOR EOSINOPHIL INFILTRATION AND SURVIVAL OF COLORECTAL CANCER PATIENTS

Anna E. Prizment[1], Robert A. Vierkant[2], Thomas C. Smyrk[2], Lori S. Tillmans[2], James J. Lee[3], P. Sriramarao[1], Heather H. Nelson[1], Charles F. Lynch[4], Steve N. Thibodeau[2], Kristin E. Anderson[1], Paul J. Limburg[2]

[1]University of Minnesota, Minneapolis, MN; [2]Mayo Clinic, Rochester, MN; [3]Mayo Clinic Arizona, Scottsdale, AZ; [4]University of Iowa, Iowa City, IA

Background: Innate immune responses have been shown to impact the course of colorectal cancer (CRC). Our goal was to examine the survival of CRC patients in relation to tissue-infiltrating eosinophils. We quantified eosinophils and their degranulation in epithelium and stroma in tumor tissues and correlated them with tumor characteristics and survival of CRC patients in the Iowa Women's Health Study (IWHS).

Methods: Paraffin-embedded tissue samples were available from 441 incident CRC cases (white post-menopausal women aged 55-69 at baseline (1986)) among IWHS participants diagnosed in 1986-2002. Tissue microarrays (up to 6 cores per person) were constructed and immunostained with a novel specific eosinophil peroxidase (EPX) antibody. Eosinophils and their level of degranulation (assessed by EPX secretion) in tumor epithelial and stromal tissues were quantified by an experienced pathologist into 4 categories: not-detected, mild, moderate and severe infiltration/complete degranulation and averaged over cores per each person using scoring algorithm developed in Protheroe et al [2009]. We used Cox regression to estimate the hazard ratio (HR) and 95% confidence interval (CI) for total and CRC-specific death in relation to each eosinophil measure after adjusting for age of diagnosis, SEER stage, tumor grade, body mass, and smoking history.

Results: During follow-up until 2011, 31% of participants died from CRC, 37% died from all other causes, whereas 32% were alive in 2011. The median follow-up was 8.4 years (range 0-25 years). The mean intraepithelial and stromal eosinophil counts (±SD) in tumor tissues were 1.43 (±0.53) and 2.26 (±0.97), respectively, and the mean tumor degranulation score was 1.21 (±0.43). The three eosinophil measures were positively correlated (spearman correlation coefficient r=0.5-0.6), and tumor stromal eosinophil count was weakly inversely correlated with age at diagnosis, stage, and grade (r=-0.11 to -0.19). Higher intraepithelial and stromal counts were associated with an ~30% decrease in total death, and stromal count was also associated with a 39% decrease in CRC death (p-trend=0.04). After summing epithelial and stromal counts in tumor tissues, the combined HRs (95% CI) in the highest category were: 0.74 (0.56-0.97) for total death (p-trend=0.03) and 0.71 (0.47-1.05) for CRC death (p-trend=0.08) compared to the lowest category. The tumor degranulation score was not related to survival of CRC participants. Additional adjustment for surgery, chemotherapy, radiation treatment, and comorbidities at baseline or during follow-up did not markedly change the observed associations. The associations were similar in proximal and distal colon and rectum.

Conclusions: The infiltration of tumors with eosinophils, especially in tumor stromal tissue, may be an under-appreciated prognostic factor in CRC.

POSTER 28

IDENTIFICATION OF THE TRANSCRIPTION FACTOR AIOLOS AS A NOVEL REGULATOR OF EOSINOPHIL TRAFFICKING

Carine Bouff[1], Kaila L. Schollaert[1], Matthew T. Weirauch[2], Artem Barski[1,3], and Patricia C. Fulkerson[1]

[1]Division of Allergy and Immunology, 2Center for Autoimmune Genomics and Etiology, [2]Division of Biomedical Informatics and Division of Developmental Biology, [3]Division of Human Genetics, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229.

Background: Dynamic gene expression is a major regulatory mechanism that directs cell differentiation. Our expression analyses of murine eosinophil progenitors (EoPs) and eosinophils revealed expression of the transcription factors Helios and Aiolos, members

of the Ikaros family of transcription factors. Importantly, Helios and Aiolos are also expressed by human eosinophils. The regulatory roles of Helios and Aiolos in eosinophil development are completely unknown.

Methods: EoPs and eosinophils were identified by surface markers using standard flow cytometry techniques. Eosinophil frequency in the small intestine was determined by counting the number of MBP-expressing cells in a defined area with 5-10 unique areas counted per mouse. Eosinophil differentiation from wild-type and Aiolos-deficient low density murine bone marrow (LDBM) cells was induced by IL-5. Chemotactic responses of mature cultured eosinophils to CCL11 and LTB4 were evaluated in vitro using 96-well 5- micron transwell plates. Actin polymerization in cultured eosinophils was measured kinetically following CCL11 stimulation via flow cytometry using a conjugated high-affinity filamentous actin probe (phalloidin). Chemotactic responses of native eosinophils in vivo were evaluated via enumeration of eosinophils by flow cytometry in the peritoneal lavage 3 hours after intraperitoneal injection of CCL11.

Results: Eosinophil frequency was modestly, but significantly, elevated in the bone marrow (2.9 ± 0.3 vs. 1.8 ± 0.2 x 104 eosinophils per 106 bone marrow cells, $P = 0.009$, n = at least 10 mice per group) and blood (78 ± 9 vs. 51 ± 8 eosinophils per microliter, $P < 0.05$, n = at least 12 mice per group) of adult Aiolos-deficient mice compared to wild-type control mice. Surface expression of CCR3 and integrin b7 was significantly lower on Aiolos-deficient eosinophils, suggesting that Aiolos may regulate expression of genes important for cell mobilization and migration. Indeed, there was a significant enrichment for genes associated with cell migration in genes that contained Aiolos binding sites in their active promoters and were induced in mature eosinophils. Importantly, eosinophil frequency was significantly lower in the intestine of Aiolos-deficient mice compared to control mice (19 ± 2 vs. 87 ± 10 eosinophils per mm2, $P < 0.0001$, n = 8-10 mice per group). In addition, IL-5-mediated eosinophil yield from cultures of Aiolos-deficient LDBM cells was significantly reduced (83%, $P < 0.05$, n = 2 independent experiments). Notably, Aiolos-deficient eosinophils had a significantly impaired chemotactic response to CCL11 and LTB4, as well as reduced CCL11-mediated actin polymerization. Native Aiolos- deficient eosinophils also displayed impaired trafficking into the peritoneum in response to CCL11 compared to wild-type controls (10.5 ± 1.4 vs. 2.7 ± 0.7 x 104 eosinophils, P =0.007, n = 3 independent experiments).

Conclusions: Collectively, our in vitro and in vivo studies have identified the transcription factor Aiolos as a novel regulator of eosinophil trafficking, likely via regulation of genes important for eosinophil mobilization from the bone marrow and migration from the blood into tissues. Studies are underway to delineate the subset of genes that are regulated by Aiolos during eosinophil development.

Grant support: This work was supported by the NIH grant K08 AI093673 and the Allergy, Asthma, Immunology Education and Research Organization (ARTrust) Faculty Development Award of the American Academy of Allergy, Asthma and Immunology. This project was also supported in part by the NIH grant P30 DK078392 (Gene and Protein Expression and Flow Cytometry Cores) of the Digestive Disease Research Core Center in Cincinnati.

POSTER 29

DIFFERENTIAL LOCALIZATION OF STAT3A AND STAT3B IN ACUTELY ACTIVATED EOSINOPHILS

Keren B. Turton[1], Frances J. Fogerty[1], Douglas S. Annis[1], Uddalak Bharadwaj[2], David J. Tweardy[2], and Deane F. Mosher[1,3]

Departments of [1]Biomolecular Chemistry and [3]Medicine, University of Wisconsin, Madison, WI, and [2]MD Anderson Cancer Center, Houston, TX, USA

Background: STAT3 has been implicated in signaling pathways that do not involve the nucleus, most compellingly perhaps in collagen-induced platelet aggregation. We hypothesized that α- and β-splice variants have distinct localizations (and functions) in cytokine-activated eosinophils undergoing shape change and polarization.

Methods: Four variant mRNAs for signal transducer and activator of transcription 3 (STAT3) are present in eosinophils due to two alternative splicing events that lead to inclusion (S) or exclusion (ΔS) of the codon for Ser701 and transactivation domains that end with 55 (α) or 7 (β) unique C-terminal residues (Turton *et al*., *PLoS One*, 10(5): e0127243). Before or after stimulation of purified human blood eosinophils with IL5 for 5 min, cells were fixed; cytospun; immunostained with monoclonal antibodies recognizing STAT3α, STAT3β, STAT3 phospho(p)Tyr705, or STAT3α pSer727; and imaged using confocal or structured illumination microscopy. Recombinant truncated STAT3 C-terminal proteins were immunoblotted to verify antibody specificity.

Results: In immunoblots, mouse monoclonal anti-STAT3α (9D8, AbCam) recognized only Sα and ΔSα C-terminal proteins whereas anti-STAT3β (Bharadwaj *et al*., *Cancers*, 2014) recognized Sβ and ΔSβ C-terminal proteins. In unstimulated eosinophils, anti-STAT3α stained the cytoplasm and cytoplasmic membrane, whereas anti-STAT3β staining was more concentrated at the cytoplasmic membrane. After stimulation, anti-STAT3α stained the tip of the nucleopod, whereas anti-STAT3β stained the cone of the nucleopod.

Ser727 was basally phosphorylated in nuclei of unstimulated cells. Nuclear pTyr705, in contrast, increased dramatically post-stimulation. Unphosphorylated STAT3 localized to the membrane of the granular region, more strikingly for STAT3β than for STAT3α.

Conclusions: Four STAT3 splice variants exist at the transcript level in eosinophils. STAT3α and β distribution differed after IL5 treatment. Phosphorylated STAT3 localizes to the nucleus post-stimulation as a transcription factor and unphosphorylated STAT3 localizes to the granular membrane, suggesting independent function. We hypothesize that the composition of splice variants as well as phosphorylation status contributes to STAT3's dichotomous roles in signaling.

Grant support: This work was supported in part by a Program Project Grant on the Role of Eosinophils in Airway Inflammation and Remodeling P01 HL088584 (PI: Nizar N. Jarjour) from the NIH-NHLBI.

POSTER 30

LIGAND-MEDIATED SIGLEC-8 INTERNALIZATION IN EOSINOPHILS IS INFLUENCED BY THE ACTIN CYTOSKELETON, TYROSINE KINASES, DYNAMIN, AND SIALYLATED CIS LIGANDS

Jeremy A. O'Sullivan[1], Daniela Janevska[1], Corwin M. Nycholat[2], James C. Paulson[2], Michael Tiemeyer[3], and Bruce S. Bochner[1]

[1]Division of Allergy-Immunology, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA, [2]Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California, USA, [3]Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia, USA

Background: Sialic acid-binding immunoglobulin-like lectin (Siglec)-8 is a human cell surface protein expressed on eosinophils, mast cells, and basophils that induces eosinophil apoptosis and inhibits mast cell mediator release. This makes Siglec-8 an ideal target for monoclonal antibody- and glycan ligand-based therapies for diseases involving these cell types. However, the dynamics of Siglec-8 surface expression, which are crucial to these targeted therapies, are poorly understood. The objective of this study was to examine Siglec-8 endocytosis, intracellular localization, and shuttling to the cell surface to determine the feasibility of Siglec-8–targeted therapies.

Methods: Using monoclonal antibodies against Siglec-8 as well as a biotinylated synthetic ligand (6'-*O*-sulfo-3'-sialyl-LacNAc-decorated 1-MDa polyacrylamide), we examined the internalization and trafficking of Siglec-8 in eosinophils by flow cytometry and confocal microscopy. Pharmacological inhibitors of intracellular signaling molecules or components of various endocytic pathways were used to identify their roles in Siglec-8 dynamics. Finally, sialidase was used to establish specificity of binding and internalization of the receptor with the synthetic ligand and determine whether sialylated *cis* ligands may influence Siglec-8 internalization pathways or alter its surface trafficking.

Results: We observed that Siglec-8 internalization in human eosinophils proceeds slowly in response to ligation with either antibody or the polyvalent synthetic ligand; about half of the surface pool of Siglec-8 is internalized in 90 minutes. This process is prevented by treatment with disruptors of actin cytoskeletal dynamics (latrunculin B and jasplakinolide), an inhibitor of tyrosine kinases (genistein), and an inhibitor of dynamin (dynasore), but not by an inhibitor of microtubule assembly (nocodazole) or a Src family kinase inhibitor at a concentration appropriate for these kinases (PP1, 100 nM). Interestingly, agents that prevent receptor internalization were also found to inhibit apoptosis due to Siglec-8 engagement. Internalized Siglec-8 was observed to form a punctate pattern within the cell confined to an intracellular compartment that has yet to be identified. Sialidase treatment of eosinophils prior to Siglec-8 ligation enhances ligand binding, suggesting the presence of masking *cis* sialylated ligand on the eosinophil surface, and proportionally promotes Siglec-8 internalization.

Conclusions: The dynamics of Siglec-8 surface expression therefore appear to be suitable for sustained targeting and may deliver drugs effectively to intracellular compartments to treat diseases including malignancies of these cell types. While the identity of the sialylated *cis* ligand is currently unknown, modulation of this interaction may improve the efficacy of Siglec-8–targeted therapeutics.

Funded by P01 HL107151.

POSTER 31

D-TYPE PROSTANOID RECEPTOR SIGNALING PROMOTES SURVIVAL BY INHIBITION OF THE INTRINSIC APOPTOSIS PATHWAY AND ACTIVATES RELATED GENE REGULATION ELEMENTS

Miriam Peinhaupt[1], Miriam Sedej[1], David Roula[1], Akos Heinemann[1]

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

Background: Prostaglandin D2 profoundly controls eosinophil effector functions during allergic reactions and is the ligand for two distinct G-protein coupled receptors, DP (D-type prostanoid receptor) and CRTH2 (chemoattractant receptor-homologous

molecule expressed on Th2 cells). Although, CRTH2 agonists mimic several effects of PGD2, they cannot account for the full range of PGD2-mediated pro-inflammatory and immunmodulatory functions. Recently, we have shown that DP is crucial for efficient CRTH2-mediated Ca^{2+} signaling which demonstrates that DP and CRTH2 can influence each other's signaling properties [1].

Up to now, the role of DP in eosinophil biology and the particular cooperation between DP and CRTH2 is incompletely understood and might be the key to fully explain the effects of PGD2 on eosinophils in allergic diseases.

This study, first, aims to address the capacity of the interlinked DP and CRTH2 signaling to induce transcriptional - and hence - functional changes in eosinophils. Second, the role of the DP receptor is controversial and its specific role in the functionality of eosinophils in the setting of allergic diseases is not clear up to now.

Methods: Human peripheral blood eosinophils and HEK293 cells overexpressing DP and/or CRTH2 were used as a two-way approach optimized for functional assays with primary cells and a system to screen for signaling pathways. Survival of eosinophils and HEK293 cells was determined by AnnexinV/PI co-staining, MTS testing and JC1 staining. Proliferation of HEK293 cells was monitored constantly by Electrical Cell Substrate Sensing (ECIS). Serum response element induction was determined by reporter gene assays.

Results: The DP mediated pro-survival effect of PGD2 on human peripheral blood eosinophils (shown by AnnexinV/PI co-staining) was reflected by protection of the mitochondrial membrane potential ($\Delta\psi m$) and inhibition of Caspase 3/7. Further, PGD2 enhanced the viability of serum-starved HEK293 cells overexpressing DP (HEK-DP and HEK-CRTH2+DP) but not of HEK-CRTH2 cells. Besides viability enhancement, DP receptor expression increased the proliferation of HEK293 cells upon treatment with PGD2. DP but not CRTH2 receptor stimulation induced SRE activation (luciferase assay) which indicated the potential of DP to regulate eosinophil homeostasis by modulation of gene expression.

Conclusions: The DP receptor profoundly complements the immediate chemotactic stimulus of CRTH2 by, first, maximizing the response to PGD2 and second, activating gene regulation which leads to inhibition of the intrinsic apoptosis pathway and hence promotes eosinophil survival. In this way the distinct effects of DP and CRTH2 complement each other and might contribute to the early and late phase of an allergic response.

1. Sedej, M. *et al.* D-type prostanoid receptor enhances the signaling of chemoattractant receptor-homologous molecule expressed on T(H)2 cells. *J. Allergy Clin. Immunol.* 129, 492–500, 500.e1–9 (2012).

POSTER 32

EOSINOPHILS UNDERGO CYCLOPHILIN D-DEPENDENT REGULATED NECROSIS

Nives Zimmermann[1,3], Xiang Zhu[1], Jeffery D. Molkentin[1], Marc E. Rothenberg[1] and Margaret H. Collins[2]

Departments of [1]Pediatrics and [2]Pathology, Cincinnati Children's Hospital, and [3]Department of Pathology, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA.

Background: Deregulated cell death characterizes many human diseases. For instance, eosinophil-associated diseases are characterized by toxic granule proteins and/or clusters of free intact extracellular granules deposited in the tissue; however, the mechanism of their release from cells is incompletely understood. Recent studies have suggested the existence of novel, distinct cell death processes such as regulated necrosis in addition to apoptotic and necrotic cell death; this form of cell death may be responsible for release of granules in eosinophil-associated diseases. Importantly, these processes are differentially regulated by biochemical cascades and can thus be targeted for therapeutic interventions in patients with eosinophil-associated diseases.

Methods: We tested the hypothesis that eosinophils undergo regulated necrosis using biopsy specimens from patients with eosinophilic esophagitis (EoE), and bone marrow (BM)-derived eosinophils from mice deficient in cyclophilin D, a protein required for mitochondrial permeability transition-mediated regulated necrosis, (gene name *Ppif)*. Eosinophil cell death in tissue was assessed by transmission electron microscopy and light microscopy. Cell death of BM-derived eosinophils was assessed by flow cytometry for annexin V staining and 7AAD uptake.

Results: Analysis of biopsy specimens from patients with EoE demonstrated eosinophils with disrupted plasma membrane, granules with reversal of granule core staining consistent with activation, and clusters of intact free extracellular granules. Since steady-state assessment in human tissue cannot determine the process by which these free granules were released, we turned to BM-derived eosinophils from *Ppif*-deficient mice. There was no difference in the development of wild type and *Ppif*-deficient eosinophils or proliferation *in vitro*. As expected, wild type eosinophils underwent necrosis following treatment with ionomycin and hydrogen peroxide, stimuli for Ca^{2+} overload and oxidative stress-induced regulated necrosis, respectively. However, compared to wild type eosinophils, regulated necrosis of *Ppif*-deficient eosinophils was decreased 44.2% and 34.9 % respectively (P=0.04 and <0.01). In

contrast, cell viability with apoptosis-inducing stimuli such as cytokine (IL-5) withdrawal, anisomycin, camptothecin and anti-Fas antibody, was not affected by the absence of cyclophilin D.

Conclusion: In addition to cell death by apoptosis, eosinophils undergo regulated necrosis. This process is mediated, at least in part, by cyclophilin D. Since this pathway is biochemically regulated, it is amenable to pharmacological manipulation, and targeting this pathway may affect disease progression in eosinophil-associated diseases.

Grant support: NIH grant AI103853 to NZ and MHC

POSTER 33

DISTINCT MODES OF REACTIVE OXYGEN SPECIES GENERATION IN EOSINOPHIL CELL DEATH INDUCED BY SIGLEC-8/IL-5 CO-STIMULATION AND EXTRACELLULAR DNA TRAP CELL DEATH

Gen Kano[1] and Nives Zimmermann[2]

[1]Department of Pediatrics, Kyoto Prefecture University of Medicine, Kyoto, Japan
[2]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Ohio, USA

Background: Recently, reactive oxygen species (ROS) were shown to play a major role in two types of lytic (granule-releasing) eosinophil cell death, namely, extracellular DNA trap cell death (ETosis) and cell death induced by Siglec-8/IL-5 co-stimulation. Importantly, eosinophils produce both extracellular and intracellular ROS. ETosis was shown to specifically require extracellular ROS; however, in Siglec-8/IL-5-induced cell death such distinction has not been examined. We conducted a comparative study of the mode of ROS generation in these two types of cell death.

Methods: Human eosinophils from peripheral blood of healthy volunteer donor were isolated by CD16 negative selection method. After isolation, eosinophils were incubated in RPMI media containing 10% fetal bovine serum (FBS), or in some experiments, RPMI with 0.1% bovine serum albumin (BSA) media to avoid known ROS–scavenging effect of FBS. Cells were incubated with cell death stimuli, i.e., Ca^{2+} ionophore A23187 for ETosis; monoclonal mouse anti-Siglec-8 antibody and recombinant human IL-5 for Siglec-8/IL-5 mediated cell death. Extracellular ROS was scavenged by addition of extracellular catalase. Amount of cell death was measured by Annexin-V/7AAD flow cytometry. Granular release upon cell death was evaluated by eosinophil peroxidase (EPX) release assay using O-phenylenediamine chemiluminescence method. To detect ETosis, cells were incubated in chambered glass slides, fixed by paraformaldehyde, stained with DAPI, and evaluated by fluorescent microscopy. Extracellular ROS generation was determined with Amplex-Red® chemiluminescence assay. Intracellular ROS generation was measured by dihydroxyrhodamine 123 (DHR) flow cytometry.

Results: A23187 and Siglec-8/IL-5 induced comparable levels of EPX release and cell death, measured after 3 hours and over-night culture, respectively. Stimulation with A23187 induced significant increase of extracellular ROS but not of intracellular ROS. This stimulation also induced nuclear morphologic changes typical to ETosis in the 0.1%BSA media. These morphologic changes were partially prevented by scavenging ROS when using 10%FBS media, and completely blocked by adding catalase, consistent with previous studies. Inhibiting effects of catalase on extracellular ROS levels and cell death was also seen after 3 hours stimulation with A23187. In contrast, stimulation of eosinophils with Siglec-8/IL-5 induced accumulation of intracellular but not extracellular ROS. Furthermore, Siglec-8/IL-5 stimulation did not induce ETosis-specific morphologic changes in neither 0.1%BSA nor 10%FBS media, and scavenging extracellular ROS with catalase did not prevent cell death induced by Siglec-8/IL-5 at any time point tested.

Conclusions: These results suggest that ETosis and Siglec-8/IL-5 activate distinct mechanism of ROS production. While extracellular ROS is required for ETosis, it is not required for Siglec-8/IL-5-induced eosinophil cell death. Considering previous studies demonstrated that ROS are required for Siglec-8/IL-5-induced cell death, our data suggest it is intracellular ROS that is involved in this form of cell death.

POSTER 35

ACTIVE EOSINOPHILIC ESOPHAGITIS IS CHARACTERIZED BY EPITHELIAL BARRIER DEFECTS AND EOSINOPHIL EXTRACELLULAR TRAP FORMATION

Dagmar Simon, Susanne Radonjic-Hösli, Alex Straumann, Shida Yousefi, Hans-Uwe Simon

Department of Dermatology, Inselspital, Bern University Hospital, Bern, Switzerland; Institute of Pharmacology, University of Bern, Bern, Switzerland; Swiss EoE Research Network, Olten, Switzerland.

Background: Eosinophilic esophagitis (EoE) exhibits esophageal dysfunction owing to an eosinophil-predominant inflammation. Activated eosinophils generate eosinophil extracellular traps (EETs) able to kill bacteria. There is evidence of an impaired barrier

function in EoE that might allow pathogens to invade the esophagus. This study aimed to investigate the presence and distribution of EETs in esophageal tissues from EoE patients and their association with possible epithelial barrier defects.

Methods: Anonymized tissue samples from 18 patients with active EoE were analyzed. The presence of DNA nets associated with eosinophil granule proteins forming EETs and the expression of filaggrin, the protease inhibitor lympho-epithelial Kazal-type-related inhibitor (LEKTI), antimicrobial peptides, and cytokines were evaluated by confocal microscopy following immune fluorescence staining techniques.

Results: EET formation occurred frequently and was detected in all EoE samples correlating with the numbers of infiltrating eosinophils. While the expression of both filaggrin and LEKTI was reduced, epithelial antimicrobial peptides (human beta-defensins-2, -3, cathelicidin LL-37, psoriasin) and cytokines (TSLP, IL-25, IL-32, IL-33) were elevated in EoE as compared to normal esophageal tissues. There was a significant correlation between EET formation and TSLP expression (p=0.02) as well as psoriasin expression (p=0.016). On the other hand, a significant negative correlation was found between EET formation and LEKTI expression (p=0.016).

Conclusion: Active EoE exhibits the presence of EETs. Indications of epithelial barrier defects in association with epithelial cytokines are also present which may have contributed to the activation of eosinophils. The formation of EETs could serve as a firewall against the invasion of pathogens.

POSTER 36

EOSINOPHIL REGULATES TRANSCRIPTIONAL EXPRESSION OF GENES INVOLVED IN VARIOUS PHYSIOLOGIC RESPONSES IN THE SMALL INTESTINE

YunJae Jung[1], Ting Wen[2], Eun-Hui Lee[1] and Marc E. Rothenberg[2]

[1]Department of Microbiology, Graduate School of Medicine, Gachon University, Incheon 406-799, Republic of Korea; [2]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA

Background: Under homeostatic conditions, most eosinophils develop in the bone marrow and migrate into the gastrointestinal tract. Though accumulation of eosinophils in the intestine occurs before birth independent of microbial stimulation, little is known about basal function of gastrointestinal eosinophils. To begin to address this vacuum in knowledge, we undertook whole genome RNA sequencing (RNA-seq) of the small intestinal tract of wild-type (WT) and eosinophil-deficient ΔdblGATA mice and interrogated whether key putative genes were expressed by intestinal eosinophils.

Methods: RNA isolated from the small intestine of WT (n=4) and ΔdblGATA (n=4) mice was subjected to RNA-seq. The aligned total sequencing reads were normalized to detect FPKM (fragments per kilobase of exon per million mapped reads) and FPKM > 0.1 in at least 1 out of 8 analyzed samples was used as a threshold to filter potentially significant gene expressions. FDR corrected P-values < 0.05 with 2-fold differences was used to screen differential gene expressions (DEG). A subset of significant DEG were chosen to perform real-time PCR (RT-PCR) to validate RNA-seq results and were compared with the mRNA microarray expression values of purified eosinophils isolated from the small intestine of WT mice. Functional groups encompassing the differential expression genes were identified based on Gene Ontology (GO) analysis.

Results: Compared to the WT small intestine, 379 down-regulated genes (including *Ccr3*, *Cebpe*, *Gata1*, *Il5ra*, and *Epx*) and 52 up-regulated genes (including *Meis2*, *Kcnip3*, *Zscan21*, *Nkx2-1*, and *Hmga1*, genes have function in the transcriptional regulation) were observed in the small intestine of ΔdblGATA mice. RT-PCR results of 20 genes, selected from those with differing expression patterns and from genes of interest based on functional analysis, confirmed the RNA-seq results (Correlation Coefficient = 0.77; P<0.0002). The expression patterns of RT-PCR validated genes partly overlapped with the mRNA microarray expressions of small intestinal eosinophils. The mRNA expressions for *Ccr3*, *Cebpe*, *Alox15*, *Serpinb2*, *Slco4c1*, *Il1b*, *Ncf1*, and *F5* were substantial in intestinal eosinophils; whereas, *Retnlg*, *Faim*, *Drd2*, *Thsd1*, *Ptger3*, *Htr2b*, *Gap43*, *Nos2*, *Stmn2*, *Tubb3*, and *Chl1* were not significant in purified intestinal eosinophils, implying both direct and indirect roles of eosinophils for the transcriptional regulation in the small intestine. The GO terms of the down-regulated genes included inflammatory and defenses responses, chemotaxis, lipid metabolism and neuron development/differentiation.

Conclusions: Eosinophils have a role in the regulation of transcript expression profiles in the small intestine under steady state. Although eosinophils have been considered to be destructive effector cells, this study suggests the potential role of eosinophils for the regulation of a series of physiologic responses in the small intestine including innate immunity, regulation of lipid metabolism and axon development. Taken together, these findings provide a foundation for future studies uncovering the functional role of eosinophils in the small intestine.

POSTER 37

SIGNIFICANCE OF FOOD SKIN PRICK TESTING IN ADULT EOSINOPHILIC ESOPHAGITIS PATIENTS

Ashleigh A. Olson, David M. Manthei, Chloe H. Kim, Sameer K. Mathur, University of Wisconsin School of Medicine and Public Health

Background: Eosinophilic Esophagitis (EoE) is associated with atopy. The significance of food sensitivity in adult EoE patients is still not well understood. We characterized patients in our allergy clinics to determine the utility of skin prick testing (SPT) in adult patients with EoE.

Methods: An IRB approved retrospective chart review was completed, identifying 724 adults with a diagnosis of EoE seen within the University of Wisconsin Health care system from 2008-2013. Patient charts were accessed for demographics, disease severity, endoscopy results, food and aeroallergen testing, management recommendations, and clinical outcomes.

Results: 256 of the 724 adult patients were seen in Allergy clinic. Average age at evaluation was 38.8 years of age and 61% were men. 81% had SPT to foods and 40% had SPT to aeroallergens. The prevalence of aeroallergen sensitization was 82%. The prevalence of food sensitization to at least one food was 51%. In patients tested to the six-food panel, the most common food sensitivity was peanut (29%) followed by soy (20%). Preliminary analyses indicate no significant differences between food sensitive and non food sensitive patients in regards to demographics, clinical characteristics, disease severity or outcomes. Approximately 1/3 of patients had symptoms for over 10 years and the most common symptoms were dysphagia, reflux and food impaction. Over 80% of patients regardless of food sensitivity improved with treatment, which most commonly included swallowed steroid (82%) and/or proton pump inhibitor (78%). Food avoidance was not related to symptom outcomes. Food sensitive patients did, however, have significantly higher eosinophil counts on esophageal biopsy than non food sensitive patients.

Conclusions: The prevalence of food and aeroallergen sensitivity in adult EoE patients is 51% and 82%, respectively. Preliminary analyses indicate significant improvement in symptoms primarily with use of swallowed steroid and proton pump inhibitor, but no relationship between patient characteristics, disease severity or symptom outcomes and sensitivity to foods. The significance of SPT in adult patients with EoE is still unclear.

POSTER 38

A KEY REQUIREMENT FOR CD300LF REGULATING INNATE IMMUNE RESPONSES OF EOSINOPHILS IN COLITIS

Moshkovits Itay[1], Reichman Hadar[1], Karo-Atar Danielle[1], Rozenberg Perri[1], Itan Michal[1], Ben-Baruch-Morgenstern Netali[1], Zigmond Ehud[2], Ziv-Haberman Yael[3], Lampinen Maria[4], Carlson Marie[4], Denson A. Lee, Varol Chen[2], Munitz Ariel[1]

[1] Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Israel, [2] Research Center for Digestive Tract and Liver Diseases, Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Israel, [3] Department of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, USA, [4] Gastroenterology Research Group, Department of Medical Sciences, University Hospital, Uppsala, Sweden.

Background: Chronic activation of innate immune cells in the colon is a key triggering event in the development of inflammatory bowel diseases (IBD). The CMRF35-like-molecule (CLM)/CD300 receptors are membrane-bound glycoprotein receptors expressed by innate immune cells which participate in cell activation and regulation. We have recently demonstrated an eosinophil-associated expression pattern for CD300lf in the colon. Yet, the role of CD300lf in IBD is unknown.

Methods: Human CD300 family members and S100a8 transcript levels were assessed in ileum biopsies of IBD and control patients (RNAseq). Wild type (WT), *Cd300a-/-*, *Cd300lf-/-*, *dblGATA* and *dblGATA/Cd300lf-/-* mice were treated with 2-2.5% dextran sodium sulfate (DSS) in their drinking water for 7 days. Weight lose, colonoscopy/clinical score, colon shortening, histopathology, inflammatory infiltration and mediator release from colon punch biopsies were examined. Monocytes were depleted using the MC-21 antibody during DSS-induced colitis. Diverse myeloid populations were sorted from the colons for qPCR analysis. Mediator secretion from *E. Coli*-activated bone marrow-derived mast cells, monocytes, neutrophils and eosinophils, was assessed by ELISA and *E. Coli*-induced signaling was examined by PhosphoFlow.

Results: RNA sequencing of 162 pediatric Crohn's disease patients revealed upregulation of multiple CD300-family members, which correlated with presence of severe ulcerations and inflammation. Increased expression of CD300-family receptors was also observed in mice following induction of experimental colitis (using DSS). Specifically, expression of CD300a and CD300lf was dynamically regulated in monocytes and eosinophils. DSS-treated *Cd300lf-/-* mice exhibit attenuated disease activity and histopathology in comparison to DSS-treated *Cd300a-/-* or WT mice. Decreased disease activity in *Cd300lf-/-* mice was accompanied with reduced inflammatory cell infiltration and nearly abolished production of pro-inflammatory cytokines. Monocyte and eosinophil depletion

experiments in *Cd300lf-/-* mice and ex-vivo activation assays revealed a cell-specific requirement for CD300lf in innate immune activation of eosinophils.

Conclusions: Our data demonstrates that CD300lf is upregulated in IBD and that it is required for development of DSS-induced colitis specifically by regulating innate immunity eosinophil activation. Collectively, we uncover a new pathway regulating innate immune activities of eosinophils, a finding with significant implications in eosinophil-associated gastrointestinal diseases.

POSTER 39

EOSINOPHILS FROM EOSINOPHILIC ESOPHAGITIS PATIENTS EXPRESS FOXP3 AND USE GALECTIN-10 TO SUPPRESS T CELLS

Jennie Andersson[1,2], Christine Lingblom[1], Madeleine Ingelsten[1,2], Mogens Bove[3], Henrik Bergquist[4], and Christine Wennerås[1,2]

[1]Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [2]Department of Clinical Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden, [3]Department of ENT, Head and Neck Surgery, NÄL Hospital, Trollhättan, Sweden, [4]Department of ENT, Head and Neck Surgery, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Background: Eosinophil esophagitis (EoE) is a chronic inflammatory disease in which the esophagus is infiltrated by eosinophils and CD3+ T cells. EoE cannot be triggered in T cell-deficient mice, suggesting the involvement of T cells in disease pathogenesis. Galectin-10, the canonical eosinophilic protein, has been shown to mediate the T cell suppressive capacity of regulatory T cells. The aim of this study was to test the hypothesis that eosinophils from EoE patients have T cell regulatory properties.

Methods: A total of 31 adult, symptomatic EoE patients were included in the study. Blood eosinophils were analyzed with regards to potential T cell regulatory characteristics. Specifically, the expression of galectin-10 and FOXP3 was determined using flow cytometry and quantitative PCR. Eosinophilic FOXP3 expression was further investigated using image flow cytometry. The capacity of eosinophils from EoE patients to diminish T cell proliferation was analyzed using a co-culture system consisting of a mixed lymphocyte reaction and eosinophils. Eosinophils from 34 healthy individuals served as control material.

Results: Blood eosinophils from EoE patients had a higher expression of galectin-10 compared to healthy controls, both in terms of galectin-10 protein (> 2-fold; $p < 0.01$) and galectin-10 mRNA (4.8-fold; $p < 0.0001$). EoE patients also had an eosinophilic FOXP3 expression that was higher, in terms of protein (1.9-fold; $p < 0.05$) and mRNA (3.6-fold; $p < 0.001$), compared to controls. Eosinophilic FOXP3 was primarily located in the cytosol, both in EoE patients and healthy controls. Further, eosinophils from EoE patients and controls differed in their T cell suppressive capacity, where 51 % of the T cell proliferation was inhibited by eosinophils from EoE patients, compared to a 90% inhibition of T cell proliferation exerted by healthy eosinophils ($p = 0.011$). Neutralization of galectin-10 partially prevented eosinophilic suppression of T cell proliferation, such that T cell proliferation was 31% higher when MLR:eosinophil co-cultures were supplemented with anti- galectin-10 antibodies ($p = 0.002$).

Conclusions: We show for the first time that eosinophils express cytosolic FOXP3 and that eosinophilic FOXP3 expression is elevated in EoE patients. Further, eosinophilic galectin-10 has T cell suppressive characteristics, in addition to being expressed at a higher level in EoE patients.

Finally, eosinophils from EoE patients can suppress T cell proliferation, albeit to a lesser extent than eosinophils from healthy controls. Based on these results, we suggest that one role for eosinophils in the esophagus of EoE patients is to attempt to regulate T cell proliferation, in a partially galectin-10-dependent manner. The function of eosinophilic FOXP3 remains to be elucidated.

Grants: This work was funded by grants from LUA-ALF #71580, Strategic ALF/Transplantation #74080, Cancer and Allergy Foundation Research grant #149781, Region Västra Götaland #96490, Inga- Britt and Arne Lundberg Research Foundation, and the University of Gothenburg.

POSTER 40

AGE-DEPENDENT DIFFERENCES IN THE MOLECULAR PATTERNS OF EOSINOPHILS FROM EOSINOPHILIC ESOPHAGITIS PATIENTS AND HEALTHY PERSONS

Christine Lingblom[1], Timo Käppi[2], Henrik Bergquist[3], Mogens Bove[4], Rikard Arkel[5], Robert Saalman[2] & Christine Wennerås[1]

[1]Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, [2]Department of Pediatrics, Institution of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, [3]Department of ENT, Head and

Neck Surgery, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, [4]Department of ENT, Head and Neck Surgery, NÄL Hospital, Trollhättan, Sweden, [5]Department of Pediatrics, NÄL Hospital, Trollhättan, Sweden

Background: It has been debated whether pediatric and adult eosinophilic esophagitis (EoE) represent different disease entities. We have previously shown that blood eosinophils from adult patients with EoE have a distinct molecular pattern. The objectives of this study were to determine if the molecular pattern of eosinophils in the blood of children with EoE is: 1) distinct from that of healthy children; and 2) different from that of adult patients with EoE.

Methods: Eosinophils from children and adults with EoE, and age-matched controls, were analyzed with flow cytometry regarding their levels of the surface markers CD23, CD44, CD54, CRTH2, and intracellular expression of FOXP3 and galectin-10. Levels of mRNA for FOXP3 and galectin-10 in eosinophils were determined by quantitative PCR. Multivariate analyses of pattern recognition were performed to determine if the children with EoE could be distinguished from the healthy children based on analyses of these molecules.

Results: An eosinophilic molecular profile capable of distinguishing children with EoE from age-matched controls was identified. A lower fraction of eosinophils from children with EoE expressed CD44 ($P<0.01$) and a larger fraction expressed CRTH2 ($P<0.01$) than the age-matched controls. In addition, eosinophils from children with EoE had higher levels of galectin-10 mRNA ($P<0.05$) and lower levels of FOXP3 mRNA ($P<0.01$). The eosinophilic molecular pattern of adult EoE patients differed from that of children with EoE in having higher levels of FOXP3 mRNA, higher levels of CD54 and a larger proportion of CD23-expressing eosinophils compared with age-matched controls. A key finding was the discovery of age-dependent differences in healthy individuals regarding the levels of several eosinophilic markers.

Conclusions: Children with EoE can be distinguished from healthy children based on the molecular patterns of their blood eosinophils, which might be exploited for diagnostic purposes. Age-related physiologic differences in eosinophilic molecular patterns may partly explain the different blood eosinophil phenotypes in adult versus pediatric EoE.

Grant support: LUA-ALF #71580, Strategic ALF/Transplantation #74080, Cancer and Allergy Foundation Research grant #149781, Region Västra Götaland #96490, Inga-Britt and Arne Lundberg Research Foundation, and the University of Gothenburg.

POSTER 41

ESOPHAGEAL IMMUNOGLOBULIN LEVELS IN EOSINOPHILIC ESOPHAGITIS ESOPHAGEAL TISSUE

Melissa K. Mingler[1], Julie M. Caldwell[1], and Marc E. Rothenberg[1]

Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Background: Based on results from elimination diets and several clinical trials, strong data suggest that eosinophilic esophagitis (EoE) is non-IgE-mediated. Recently, Clayton et al. (2014, Gastroenterology) reported that adult EoE patients had higher levels of IgG4 in esophageal tissue. They additionally found that the IgG4 was localized to the deep lamina propria of the esophagus. Herein, we aimed to determine if the pediatric EoE population exhibited a similar pattern of esophageal immunoglobulin levels.

Methods: Esophageal biopsies were obtained from pediatric patients with EoE (≥15 eosinophils/HPF) and control patients, all of Caucasian descent. The biopsies were stored in RNA later (-80°C) until protein isolation and quantitation were performed in the presence of Roche Complete Protease inhibitors. All samples were diluted to equivalent total protein concentrations (100 mg/ml). The IgA, IgM, IgG1, IgG2, IgG3, and IgG4 were measured using the Luminex 100 system (Millipore) and IgE was quantified by ELISA (Alpco). All data were normalized (mg immunoglobulin/ g total protein). IgE data are expressed as IU/ml.

Results: No significant difference in average age was observed between patient populations (controls, 7.8 ± 1.2 years vs. patients with EoE, 9.2 ±1.2 years). IgG1 (controls, 35.43 ± 11.75 vs. patients with EoE, 47.02 ± 18.86 mg/g protein), IgG2 (controls, 30.92 ± 12.87 vs. patients with EoE, 46.80 ± 19.29 mg/g protein), IgG3 (controls, 0.58 ± 0.54 vs. patients with EoE, 2.47 ± 1.87 mg/g protein), and IgE (controls, 4.687 ± 0.28 vs. patients with EoE, 4.55 ± 0.30 IU/ml) exhibited similar levels in EoE biopsies and control biopsies. IgA and IgM were not detectable by this method. A significant increase in the tissue levels of IgG4 in the biopsies of EoE patients was observed compared to controls (mean IgG4 in control tissue, 0.44 mg/ml vs. EoE tissue, 10 mg/ml; $P < 0.001$). An increase in the percent of IgG4 relative to total IgG was observed in the EoE biopsies compared to control tissue (mean percentage in control tissue 2 ± 1.87% vs. 8.25 ± 5.48% in EoE tissue [$P < 0.02$]). No significant correlations were exhibited based on age, sex, or usage of glucocorticoids, proton pump inhibitors, or H1/H2 blockers.

Conclusions: In contrast to the other major Ig isotypes, IgG4 levels in pediatric EoE biopsies are increased.

Grant support: This work was supported in part by NIH R37 AI045898, U19 AI070235, U19 AI066738 (CoFAR supported by NIAID and NIDDK), R01 DK076893, R01 AI057803**,** P30 DK078392 (Gene and Protein Expression Core), U54 AI117804 (CEGIR supported by NCATS, NIAID and NIDDK), the Campaign Urging Research for Eosinophilic Disease (CURED), the Buckeye Foundation, and Sunshine Charitable Foundation" and its supporters, Denise A. Bunning and David G. Bunning.

POSTER 42

EOSINOPHIL GRANULE PROTEINS IN STOOL: A POTENTIAL NON-INVASIVE BIOMARKER OF EOSINOPHIL INFILTRATION IN GUT TISSUE

Michelle A. Makiya[1], Elise M. Ferre[2], Tamika S. Magee[3], Thomas B. Nutman[4], Calman Prussin[5], Michail S. Lionakis[2] and Amy D. Klion[1]

[1]Human Eosinophil Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, [2]Fungal Pathogenesis Unit, Laboratory of Clinical Infectious Disease, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, [3]Clinical Parasitology Section, Laboratory of Parasitic Diseases, Clinical Research Directorate/CMRP, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD [4]Helminth Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, [5]Mast Cell Biology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Background: Peripheral blood absolute eosinophil counts for eosinophilic gastrointestinal disorders (EGID) are often within the normal range (<0.5 x 10^9/L) despite eosinophilic infiltration of gastrointestinal tissue. Therefore, diagnosis and verification of treatment effectiveness are determined by endoscopy with tissue biopsy. In an attempt to find an alternative to this invasive technique, eosinophilic granule protein levels were evaluated in serum and stool samples from normal donors and subjects with gastrointestinal eosinophilia.

Methods: Stool and serum samples were collected from normal donors and subjects with EGID (serum only; n=11), parasitologically-confirmed strongyloidiasis (STRONGY; n=14) or autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED; n=27). Stool samples were weighed, diluted, homogenized and centrifuged to acquire stool supernatants. All samples were reduced and alkylated prior to performing a suspension array assay in multiplex specific for the eosinophil granule proteins major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO). To verify that detection of eosinophil granule proteins in stool is not inhibited or bound by other factors, supernatants from APECED patient stool were assayed with or without the addition of 50 ng/ml of purified eosinophil granule proteins.

Results: As reported previously, geometric mean (GM) serum levels of all 4 granule proteins were increased in subjects with biopsy-proven EGID compared to normal controls. Although GM serum MBP, EDN, and EPO levels were also significantly increased in STRONGY subjects, GM serum levels of ECP in these subjects were significantly decreased compared to normal donors (675 vs. 1555 ng/ml, $p = 0.03$). A similar pattern was seen in stool supernatants of those with STRONGY with increased GM levels of MBP (220 vs 20 pg/g stool, $p = 0.03$), and EPO (1640 vs. 7 pg/g stool, $p < 0.001$), when compared to supernatants from normal individuals.

In view of histopathologic findings demonstrating eosinophilic infiltration in GI biopsies from some patients with APECED, stool and serum levels of eosinophil granule proteins were assessed in a cohort of APECED patients with and without GI symptoms. Surprisingly, GM serum levels of MBP, EDN and EPO were significantly decreased in APECED patients compared to normal donors, although many of the subjects were receiving hydrocortisone replacement therapy at the time the samples were collected due to adrenal insufficiency. Although no significant differences were identified in GM levels of stool eosinophil granule proteins between APECED patients and normal donors, EPO and MBP levels were markedly elevated (100-1000 times the GM level in normal stool) in a subset of the APECED patients. Of note, stool levels of ECP were significantly correlated with stool calprotectin levels ($r = 0.43$, $p = 0.03$), stool levels of EDN were correlated with peripheral eosinophil count ($r = 0.51$, $p < 0.01$) and fecal fat score ($r = 0.41$, $p = 0.03$), and both stool ECP and EDN showed a trend towards a positive correlation with diarrhea score in patients with APECED.

Conclusions: Eosinophil granule proteins can be measured in stool supernatants by multiplex assay and may provide a better marker of eosinophilic infiltration in the gut than serum assays. Correlation with histopathologic findings is currently underway.

Grant support: This study was funded by the Division of Intramural Research, NIAID, NIH. Funded by the NCI Contract No. HHSN261200800001E. This research was supported in part by the National Institute of Allergy and Infectious Diseases.

POSTER 43

IL-33 IS SELECTIVELY EXPRESSED BY ESOPHAGEAL EPITHELIAL PROGENITOR CELLS DURING ALLERGIC INFLAMMATION

Jared Travers[1], Mark Rochman[1], and Marc E. Rothenberg[1]

Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Background: Recent studies on the pathogenesis of allergic disorders have focused on the involvement of innate cytokines produced by epithelial cells that promote the development of Th2 cell immunity. Herein, we focused on the involvement of the innate cytokine IL-33 in eosinophilic esophagitis (EoE). We aimed to test the hypothesis that IL-33 is increased in EoE.

Methods: Quantitative real-time PCR (qRT-PCR), immunohistochemistry (IHC) and immunofluorescence (IF) were performed on esophageal biopsies of patients with inactive and active EoE or control individuals.

Results: We noted a profound alteration in IL-33 protein expression in the diseased state as only patients with active disease had detectable epithelial expression of IL-33; expression was limited to the nuclei of the cellular layer in direct contact with the basement membrane between papillae. Consistent with this, IL-33 mRNA was increased (2-fold; $p = 0.045$) in active EoE biopsies compared to control biopsies. IL-33$^+$ cells expressed E-cadherin, keratin 5 and keratin 14 (basal layer epithelial markers), and the epithelial progenitor markers p75NTR and podoplanin. They did not express the proliferation marker Ki-67. Additionally, a subset of primary esophageal epithelial cells grown *in vitro* expressed IL-33 but not Ki-67.

Conclusions: IL-33 is selectively present only during active EoE disease in the most basal layer in a quiescent progenitor population. We propose that IL-33 is likely involved in disease pathogenesis and may have a role in epithelial progenitor cell biology.

Grant support: This work was supported in part by NIH R37 AI045898, U19 AI070235, U19 AI066738 (CoFAR supported by NIAID and NIDDK), R01 DK076893, R01 AI057803**,** P30 DK078392 (Gene and Protein Expression Core), U54 AI117804 (CEGIR supported by NCATS, NIAID and NIDDK), the Campaign Urging Research for Eosinophilic Disease (CURED), the Buckeye Foundation, and Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning.

POSTER 44

CLAUDIN-7 DYSREGULATION IN PEDIATRIC EOSINOPHILIC ESOPHAGITIS: A ROLE FOR TGF-B1 IN ESOPHAGEAL EPITHELIAL BARRIER DYSFUNCTION

Shahan D. Fernando[1,2], Kathryn Biette[1,2], David Kitzenberg[2], Louise E. Glover[2], Sean P. Colgan[2], Glenn T. Furuta[1,2], Joanne C. Masterson[1,2].

[1]Gastrointestinal Eosinophilic Diseases Program, Digestive Health Institute, Children's Hospital Colorado, [2]Mucosal Inflammation Program, University of Colorado, CO, USA.

Background: Altered barrier function has recently been implicated in the pathophysiology of eosinophilic esophagitis (EoE). Transforming growth factor beta (TGF-β1), a potent cytokine involved in fibrosis and remodeling, is increased in EoE, but its role in esophageal barrier function is not certain. We hypothesized that TGF-β1 leads to esophageal epithelial barrier dysfunction. The purpose of this study was to determine mechanism(s) by which TGF-β1 alters epithelial barrier.

Methods: To examine the impact of TGF-β1 on esophageal epithelial barrier, we used a 3-dimensional air-liquid interface model (3D-ALI) that induces stratification and differentiation of epithelial cells *in vitro*. Non-transformed immortalized human esophageal epithelial cells (EPC2-hTERT) were exposed to rhTGF-β (5-10 ng/mL) during the 3D-ALI process of differentiation and stratification. Functional assays of barrier were performed using transepithelial electrical resistance (TEER) and 3kDa FITC-dextran paracellular flux (FITC-Flux). Epithelial barrier (occludin, claudin-1, -4, -7, -12 and -17, E-cadherin, ZO-1, desmoglein-1, -2, and -3) molecule expression were analyzed using real time RT-PCR. Selected molecules were then evaluated in human esophageal biopsies from control and pediatric EoE subjects. Claudin-7 (CLDN7) stable knockdown cells were generated by lentiviral delivery of sh-RNA interfering constructs. Knockdown was verified by western blot and PCR and these cells were then subjected to assessments of barrier function *in vitro*.

Results: TGF-β1 exposure throughout 3D-ALI-induced epithelial differentiation and stratification resulted in barrier dysfunction with 40.4% decreased TEER ($p<0.05$) and 1.8-fold increased FITC-Flux ($p<0.05$). TGF-β1 significantly attenuated claudin-7 mRNA expression in an establishing epithelial barrier (55% decrease; $p<0.01$) compared to unstimulated control cells. TGF-β1 also significantly attenuated claudin-7 protein expression in EPC2-cells (61% decrease $p<0.05$, TGF-b1 5ng/ml; 58% decrease; $p<0.05$, TGF-b1 10ng/ml) compared to unstimulated control cells. Furthermore, knockdown of CLDN7 in esophageal epithelial cells resulted in barrier dysfunction with 37% decreased TEER and 1.6-fold increased FITC-Flux compared to sh-control cells. Finally, Claudin-7 expression

was also significantly decreased in human esophageal biopsy specimens from EoE subjects with active disease compared to those with inactive EoE (68.7% decrease; $p<0.05$) and control subjects (69.6% decrease; $p<0.01$).

Summary: TGF-β1 contributed to esophageal barrier dysfunction, inhibiting the establishment of a stratified and differentiated epithelial barrier. TGF-β1 exposure attenuated the expression of the tight junction molecule claudin-7, whose expression is also dysregulated in active EoE subjects.

Conclusion: TGF-β1 plays a role in esophageal epithelial barrier dysfunction. We anticipate that further investigations will permit identification of TGF-β1 and claudin-7's mechanisms in inducing barrier dysfunction in EoE.

POSTER 45

CDH26 IS AN INTEGRIN-BINDING IMMUNOMODULATOR INVOLVED IN ALLERGIC INFLAMMATION

Julie M. Caldwell[1], Margaret H. Collins[2], Katherine A. Kemme[1], Joseph Sherrill[1], Ting Wen[1], Mark Rochman[1], Emily M. Stucke[1], Lissa Amin[1], Haitong Tai[1], Philip E. Putnam[3], Aleksey Porollo[4], J. Pablo Abonia[1], Marc E. Rothenberg[1]

[1]Division of Allergy and Immunology, [2]Division of Pathology, [3]Division of Gastroenterology, Hepatology, and Nutrition, [4]Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: We identified a novel cadherin (CDH26) as one of the top genes markedly overexpressed in human allergic gastrointestinal inflammatory tissue. Herein, we aimed to elucidate the expression pattern and function of CDH26.

Methods: Global transcript analysis identified genes differentially expressed in gastric tissue of EG patients compared to control individuals. Further characterization of *CDH26* expression patterns was undertaken through real-time PCR, immunohistochemistry, and western blot analysis. Protein interactions were examined using transient transfection and immunoprecipitation analysis. Aggregation and binding assays assessed cellular adhesion. Human CD4+ T cell activation was monitored by assessment of cell surface protein expression by flow cytometry and measurement of cytokine secretion by ELISA.

Results: Gene ontology and pathway analysis showed that the EG transcriptome was enriched in transcripts related to immunological and metabolic pathways. *CDH26,* the most highly overexpressed transcript in EG biopsies (20.9-fold, $p<0.01$), was the only cadherin differentially regulated in EG and EoE. Similar to EoE, IL-13, which was markedly overexpressed in EG patient gastric tissue (375-fold, $p<0.01$), induced *CDH26* expression in GI epithelial cells *in vitro*. CDH26 mediated calcium-dependent cellular adhesion, exhibited homotypic interaction, and additionally interacted with beta-, alpha-, and p120-catenins. Moreover, CDH26 interacted with integrins α4 and αE. Jurkat T cells, which express α4β1, bound CDH26-hIgG1-Fc in an integrin α4-dependent manner. Activation of $CD4^+$ T cells by anti-CD3 antibodies was inhibited when the cells were cultured in the presence of plate-bound CDH26-hIgG1-Fc, as assessed by expression of cell surface molecules indicative of T cell activation as well as secretion of IL-2 and IL-4.

Conclusions: We have identified CDH26 as an inducible cadherin and receptor for select alpha integrins and that CDH26-Fc is an inhibitor of human T cell activation.

Grant support: This work was supported in part by NIH R37 AI045898, U19 AI070235, U19 AI066738 (CoFAR supported by NIAID and NIDDK), R01 DK076893, R01 AI057803, P30 DK078392 (Gene and Protein Expression Core), U54 AI117804 (CEGIR supported by NCATS, NIAID and NIDDK), the Campaign Urging Research for Eosinophilic Disease (CURED), the Buckeye Foundation, and Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning.

POSTER 46

ALVEOLAR MACROPHAGES INITIATE INFLAMMATORY RESPONSES TO *LACTOBACILLUS PLANTARUM* IN THE MOUSE AIRWAY

Tyler A. Rice, Caroline M. Percopo, Kimberly D. Dyer, and Helene F. Rosenberg

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

Background: Respiratory virus infections with rhinoviruses, influenza viruses, and paramyxoviruses all exacerbate allergic inflammation in the airways (Zhao, J, *et al.* 2002 Sigurs, N, *et al.* 2010). We have shown that immunobiotic *Lactobacillus* sps. administered directly to the respiratory mucosa can suppress the negative sequelae of acute respiratory virus infection in natural infection models *in vivo* (Gabryszewski, *et al.* 2011, Garcia-Crespo, *et al.* 2013, Percopo, *et al.* 2014). Alveolar macrophages (AM) are the predominant innate immune cell in the airways and act as first responders to clear dust or foreign microorganisms like *Lactobacillus*, and also

serve as antigen presenting cells to inform adaptive immune cells of more serious infections (Hussell, T, *et al.* 2014). Explorations of immunomodulatory *Lactobacillus* may reveal ways to combat both respiratory viruses and allergic airway inflammation.

Methods: Alveolar macrophages were isolated from C57BL/6, NOD2 and TLR2 gene-deleted mice and challenged with *L. plantarum* and other stimuli. RNA transcripts were quantified by qRT-PCR, soluble proteins by ELISA, and single cell characteristics by flow cytometry.

Results: Surface marker phenotype $CD45^{+}CD11c^{+}SiglecF^{+}$, distinct from $F4/80^{+}CD11b^{+}$ peritoneal macrophages and consistent with lung alveolar macrophages, is recapitulated in adherence-plated AM, rendering these cells a useful platform for functional studies. Isolated AM respond to *L. plantarum* challenge by expressing CXCL2, CXCL10, CCL2, IL-6, and TNF-α at 5-20 -fold over unstimulated controls, all independent of the pattern recognition receptors NOD2 or TLR2. Immunomodulation in the whole tissue, however, is characterized by suppression of part of this cytokine response: AM isolated after two doses of intranasal *L. plantarum*, express similar amounts of CXCL10, CCL2, and IL-6, but far less TNF-α, compared to AM challenged with *L. plantarum ex vivo*.

Conclusions: Alveolar macrophages rapidly initiate a significant and complex inflammatory response to *L. plantarum* in the airways by producing a number of chemokines and cytokines. Observing a suppressed macrophage TNF-α response after *in vivo L. plantarum* priming, we are led to hypothesize that lung-specific interactions and possibly epithelial-derived signals dampen macrophage production of those cytokines that might have greater potential to damage the host airway.

Funding: NIAID DIR (AI000943 to HFR)

POSTER 47

EXTRACELLULAR DNA TRAPS IN BRONCHIAL SECRETION FROM ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Shigeharu Ueki[1], Yuta Kikuchi[1], Yasunori Konno[1], Ayumi Omokawa[1], Tomoo Saga[1], Masahide Takeda[1, 2], Kazuhiro Sato[2], Masaaki Sano[2], Makoto Hirokawa[1], Peter F. Weller[3]

[1] Department of General Internal Medicine and Clinical Laboratory Medicine, Akita University Graduate School of Medicine, Akita, Japan, [2] Department of Cardiovascular and Respiratory Medicine, Akita University Graduate School of Medicine, Akita, Japan
[3] Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Background: Human neutrophils and eosinophils release filamentous chromatin structures (DNA traps) through extracellular DNA trap cell death (ETosis). Excess formation of neutrophil DNA traps has been shown to increase the viscosity of secretions in patients with cystic fibrosis. The presence of DNA traps in eosinophilic lung diseases is not well known.

Case Report: We present the case of 64-year-old Japanese man with a 10-year history of asthma referred to our hospital for cough, sputum, and chest pain that began 6 months before admission. The patient had an elevated blood eosinophil count (1,120 / microL) and serum total IgE (1,628 IU/mL). Specific IgE against Aspergillus was positive. Pulmonary function tests showed reduced forced expiratory volume in 1 sec/forced vital capacity ratio (FEV1%) of 67.9 %. Chest CT showed infiltration in the bilateral middle lobes with central bronchiectasis. Fiber-bronchoscopy revealed eosinophilic mucus plugging in the middle lobe bronchus. Collectively, the patient was diagnosed with allergic bronchopulmonary aspergillosis (ABPA). The bronchial secretion was microscopically determined to contain Charcot-Leyden crystals, numerous lytic eosinophils with free eosinophil granules. Fibrous structures were also present, which were stained with histone H1 Ab and DNA dyes, indicating the nuclear-derived DNA traps. After systemic steroid treatment, his clinical symptoms improved and a decrease in DNA traps in the bronchial secretion was observed.

Conclusions: Eosinophil ETosis-derived DNA traps were abundantly present in the bronchial secretion from a patient with ABPA, potentially contributing to its increased viscosity.

Grant support: Grant-in-Aid for Scientific Research (SU). NIH R37-AI020241 and R01-AI051645 (PFW).

POSTER 48

CORRELATION BETWEEN CCL26 PRODUCTION BY HUMAN BRONCHIAL EPITHELIAL CELLS AND AIRWAY EOSINOPHILS: INVOLVEMENT IN PATIENTS WITH SEVERE EOSINOPHILIC ASTHMA

Marie-Chantal Larose, Jamila Chakir, Anne-Sophie Archambault, Philippe Joubert, Véronique Provost, Michel Laviolette and Nicolas Flamand. Centre de recherche de l'IUCPQ, Faculté de médecine, Université Laval, Québec, QC G1V 4G5, Canada.

Background: High pulmonary eosinophil counts are associated with asthma symptoms and severity. Bronchial epithelial cells (BECs) produce CC chemokines, notably CCL26 (eotaxin-3), which recruits and activates eosinophils from asthmatic patients. This suggests that CCL26 production by BECs might be involved in persistent eosinophilia in patients with severe asthma despite a

treatment with high corticosteroid doses. We sought to determine whether CCL26 levels correlate with eosinophilia and asthma severity.

Methods: Human CC chemokines expression was assessed by means of quantitative PCR or quantitative PCR array in vehicle- or IL-13–treated BECs. CCL26 was quantitated by means of ELISA. Immunohistochemistry analyses of CCL26 and major basic protein were done on bronchial biopsy specimens.

Results: IL-13 selectively induced CCL26 expression by BECs. This increase was time-dependent and more prominent in BECs from patients with severe eosinophilic asthma ($p<0.01$). CCL26 levels measured in supernatants of IL-13–stimulated BECs also increased with asthma severity as follows: patients with severe eosinophilic asthma > patients with mild asthma ($p=0.03$) ≈ healthy subjects ($p=0.009$). Immunohistochemistry analyses of bronchial biopsies confirmed the increased levels of CCL26 in the epithelium of patients with mild and those with severe eosinophilic asthma ($p=0.027$). Tissue eosinophil counts did not correlate with CCL26 staining. However, sputum CCL26 levels significantly correlated with sputum eosinophil counts ($R2=0.60$, $p<0.0001$), suggesting that CCL26 participates in the movement of eosinophils from the tissues to the airway lumen.

Conclusions: These results show a relation between CCL26 production by IL-13–stimulated BECs, sputum eosinophil counts, and asthma severity. They also suggest a role for CCL26 in the sustained inflammation observed in patients with severe eosinophilic asthma and reveal CCL26 as a potential target for treating patients with eosinophilic asthma that are refractory to classic therapies (ML and NF equally contributed to this work).

Grant support: supported by a grant to ML and NF from the Fondation de l'IUCPQ and the J-D- Bégin Research Chair, Scholarships to MCL from La fondation Wilbrod-Bhérer et Joseph- Demers de l'Université Laval, la direction de la recherche universitaire de l'IUCPQ and the CIHR - Quebec Respiratory Health Training Program. NF was supported by a salary award from the Fonds de la recherche du Québec-Santé.

POSTER 49

TYPE 2 CYTOKINE-PRODUCING INNATE LYMPHOID CELLS (ILC2) IN BRONCHOALVEOLAR LAVAGE (BAL) AND BLOOD FROM HUMAN ASTHMA AND THEIR CONTEXT-DEPENDENT RESPONSE TO GLUCOCORTICOIDS

Sucai Liu, Yingfang Song, Dimple Shah, Weimin Liu, James T. Good, Donald Rollins, Magdalena M. Gorska, Richard J. Martin and Rafeul Alam

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

Rationale: ILC2 represent an important source of type 2 cytokines (IL5 and IL13). Their frequency in the airways and their relative contribution to IL5 production as compared to Th2 cells in human allergic asthma and rhinitis is unknown. Further, the response of ILC2 to glucocorticoids has not been studied.

Methods: We performed bronchoscopy, biopsy and BAL in asthmatic patients and disease controls. The frequency of type 2 cytokine-producing ILC2 and T cells in BAL and blood was studied ex vivo and after culture by flow cytometry.

Results: We identified BAL ILC2 as CD45+lin-FceRI-IL7Rα+IL33R+ cells. Peripheral blood ILC2 cells were identified as lin-FceRI-IL7Rα+CRTH2+ cells. ILC2 cells were positive for GATA3. They expressed IL5, IL13, IL10 and, to a lower extent, IL4. Blood ILC2 cells expressed the inhibitory receptors CD161 and KLRG1, which were down regulated upon stimulation. The expression of these inhibitory receptors was lower on BAL ILC2 indicating their heightened activation state. The persistence of the phenotype of blood derived ILC2 depended on the presence of CD4 T cells in the culture. Their depletion largely eliminated CRTH2+ cells, which were partially restored by IL2, IL7 and IL33. IL25 and TSLP could substitute for IL33. The frequency of ILC2 in BAL was significantly ($P=0.04$) higher in asthma (1.2±0.24%, N=16) as compared to disease controls (0.2±0.04%, N=12). The mean frequency of IL5+ and IL13+ cells in the ILC2 population ex vivo were 33% and 31%, respectively. The frequency of blood IL5+ILC2 was 2-fold higher in allergic asthma as compared to allergic rhinitis (0.1% vs 0.05%, respectively). The frequency of blood IL5+ T cells as compared to IL5+ILC2 was 5-fold higher in asthma and 6 fold higher in allergic rhinitis. BAL IL5+ ILC2 positively correlated ($r=0.51$, $P=0.02$) with BAL but not blood eosinophil counts. Interestingly, IL5+T cells but not IL5+ ILC2 correlated with the blood eosinophil count.

Dexamethasone and budesonide inhibited freshly isolated and in vitro generated CRTH2+ as well as IL5+ ILC2. However, the effect of glucocorticoids on ILC2 was dichotomous. Treatment of ILC2 with IL2/IL7/IL33 abrogated the inhibitory effect of dexamethasone. More importantly, the combination of the cytokine combo and dexamethasone actually stimulated the generation of ILC2. Dexamethasone upregulated the expression of IL7Rα, a known ILC2-promoting receptor. The subsequent action of IL7 facilitated the generation of ILC2.

Conclusions: The frequency of ILC2 in BAL and blood was increased in asthmatic patients. Correlation studies suggest that ILC2 controls eosinophils in the airways. In contrast, IL5+ T cells regulate blood eosinophilia. The phenotype and activity of ILC2 require support from CD4 T cells. The effect of glucocorticoids on ILC2 was context dependent. Dexamethasone inhibited ILC2 in the

absence of growth-inducing cytokines. However, in presence of the growth-inducing cytokines, especially IL7, dexamethasone stimulated ILC2 generation. Thus, the cytokine/glucocorticoid-induced ILC2 could explain persistence of airway eosinophilic inflammation in steroid-resistant asthmatic patients.

Grant support: NIH grants RO1 AI091614, RO1 AI102943 and U19 AI100275.

POSTER 50

MECHANISMS INVOLVED IN THE EXPRESSION OF CCL26 BY BRONCHIAL EPITHELIAL CELLS: IMPORTANCE IN ASTHMA AND ITS SEVERITY

Anne-Sophie Archambault, Marie-Chantal Lar ose , Véronique Provost, Jamila Chakir, Michel Laviolette and Nicolas Flamand. Centre de recherche de l'IUCPQ, Faculté de médecine, Université Laval, Québec City, QC G1V 4G5, Canada.

Background: High pulmonary eosinophil counts correlate with asthma severity and exacerbation. We recently demonstrated that sputum CCL26 levels correlate with sputum eosinophils. In the bronchial tissue, CCL26 staining is mainly localized in the epithelium and we found that among all CC chemokines, IL-13 selectively induced CCL26 expression by bronchial epithelial cells (BECs). Further, this phenomenon is significantly enhanced in BECs from severe eosinophilic asthmatics. We thus postulated that the superior CCL26 production we observed in severe asthma was the consequence of increased signaling events mediated by the IL-13 receptor. The signaling events mediated by IL-13 begin with the binding of IL-13 to a receptor consisting of the IL-4Rα and IL-13Rα1 subunits. This binding activates the transcription factor STAT6 that activates CCL26 gene transcription. Of note, STAT6 activation can be dampened by SOCS1 and SOCS3. We thus assessed the expression and functional responses of the different signaling effectors linked to the IL-13 receptors in BECs from healthy subjects, mild asthmatics, and severe eosinophilic asthmatics.

Methods: Human primary BECs isolated from bronchial biopsies were obtained from healthy subjects and subjects with mild or severe asthma presenting high level of eosinophil counts in their induced sputum. BECs were stimulated with IL-13 or vehicle for different times. Inhibitors or their vehicles were added to BECs 1 hours before IL-13. CCL26, IL-4Rα and IL-13Rα1 expressions were assessed by qPCR and CCL26 level was quantitated by ELISA in BECs supernatants. STAT6 and phosphorylated (p)STAT6 were quantitated by ELISA in BEC lysates. SOCS1 and SOCS3 level were assessed by immunoblotting.

Results: The mRNA levels of the IL-4Rα and IL-13Rα1 subunits were comparable in BECs of the three groups. We next confirmed the involvement of STAT6 in the induction of CCL26 expression by treating BECs with increasing STAT6 inhibitor AS1415499. This led to a concentration-dependent inhibition of CCL26 expression (mRNA) and production (protein) 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 decreased SOCS proteins, given that our preliminary data indicates that SOCS3 protein levels are lower in BECs from severe eosinophilic asthmatics.

Conclusions: Our results show that STAT6 is a key player for CCL26 expression and production by stimulated BECs. They also illustrate the importance of STAT6 and the implication of SOCS proteins in the enhanced CCL26 expression and production by BECs from severe eosinophilic asthmatics (*ML and NF equally contributed to this work*).

Grant support: supported by a grant to ML and NF from the Fondation de l'IUCPQ and the JD- Bégin Research Chair, and scholarships to MCL from La fondation Wilbrod-Bhérer et Joseph- Demers de l'Université Laval, la direction de la recherche universitaire de l'IUCPQ and the CIHR-Quebec Respiratory Health Network Training Program. NF was supported by a salary award from the Fonds de la recherche du Québec-Santé.

POSTER 51

A NOVEL MECHANISM FOR VIRUS-INDUCED ASTHMA EXACERBATION: IMMUNE MEMORY TO VIRUS CAN INDUCE AIRWAY HYPERREACTIVITY IN ALLERGIC ANIMALS.

Christopher Skappak PhD[1], Ramses Ilarraza PhD MSc[2], Ying-qi Wu [1], David B Jacoby MD[3], Matthew G Drake[3] and Darryl J Adamko MD FRCPC[1, 4]

Department of Pediatrics, University of Alberta, Edmonton, Alberta[1] and University of Saskatchewan, Saskatoon, Saskatchewan[2], Division of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland Oregon[3].

Background: Asthma exacerbation is most often triggered by respiratory viruses. A large number of asthmatics have airway eosinophilia, which correlates with asthma exacerbation. Prevention of asthma exacerbation is strongly associated with the inhibition of inflammation, but not prevention of virus infection. In vitro, we reported that viruses can activate eosinophils through virus-specific memory T cells. People with asthma develop virus memory after years of virus exposure. We hypothesized that virus-specific

memory could induce airway hyper-reactivity (AHR) *in vivo* through eosinophil activation, and that this would not be dependent on virus infection.

Methods: Using guinea pigs, we created an animal model of asthma that had immune memory to parainfluenza virus (PIV), but also eosinophilia from ovalbumin sensitization and challenge (OVA). We studied immune memory, AHR, airway inflammation/histology, and viral titres.

Results: Virus specific immune memory was confirmed using a T cell proliferation assay (n=3). Live PIV induced AHR and airway inflammation in both OVA and non-OVA animals (n=5 each). In contrast to non-allergic animals, OVA animals with immune memory to PIV developed AHR and airway inflammation when re-exposed to UV-inactivated virus (n=5). Non-allergic animals with PIV-memory did not develop AHR in response to UV-inactivated PIV (n=5). In OVA animals with immune memory to PIV, pre-treatment with dexamethasone or anti-IL5 (i.p., n=5 each) prevented the development of AHR in response to UV-inactivated PIV. Pre-treatment with dexamethasone or anti-IL5 (i.p., n=5 each) also decreased the development of AHR in animals exposed to live PIV, but this effect was only seen in the OVA animals.

Conclusions: We suggest for the first time that the degree of virus infection is not the major factor in virus-induced exacerbation. Instead, our data suggest that the degree of airway inflammation and eosinophilia prior to virus infection is important. Such inflammation can be triggered by immune memory to virus antigens and induce airway dysfunction.

Funding sources: The AllerGen NCE, WCHRI

POSTER 52

EOSINOPHILS DISPLAY DAMAGING AND PROTECTIVE PROPERTIES UPON VIRAL EXPOSURE; ITS RELEVANCE IN VIRUS-INDUCED LOSS OF ASTHMA CONTROL

Yanaika S. Sabogal Piñeros[1,2,$], Suzanne M. Bal[1,2,$,3], Annemiek Dijkhuis[1,2], Barbara S. Dierdorp[1,2] and René Lutter[1,2]

[1]Department of Experimental Immunology, [2]Department of Respiratory Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands, [3]current address: Department of Cell Biology and Histology.
$ equal contribution

Background: Respiratory viral infections enhance the recruitment and accumulation of eosinophils in the airways of asthma patients, which is assumed to contribute to allergic inflammation. Recent findings, however, demonstrated that eosinophilic granular constituents exert antiviral activity, suggestive of protective properties of eosinophils. Here we report that eosinophils display direct anti-viral properties.

Methods: Respiratory syncytial virus (RSV) and influenza virus (A/PR) were labeled with a fluorescent lipophilic dye (DiD) that allowed us to follow interactions with eosinophils *in vitro* and *in vivo*, by both confocal microscopy and flow cytometry. Eosinophil activation was assessed by both flow cytometry (cell surface markers) and ELISA (mediators and granular products). Mouse eosinophils from broncho alveolar lavage fluid (BALF) and lymph nodes were studied from HDM-sensitized (3 x 25µg on three subsequent days followed by 4 x 6.25µg challenges at days 14, 15, 18 and 19) and IL-5 transgenic mice (Lee et al, JI 1997, 158:1332-44). Human eosinophils were purified using negative selection (EasySep) from peripheral blood of healthy donors and in some cases from BALF of patients, before and after virus challenge.

Results: HDM-sensitized and IL-5 transgenic mice, both with enhanced airway eosinophils recovered more rapidly from an influenza infection compared to appropriate controls. Four hours after challenge, fluorescently-labelled influenza had associated with eosinophils in broncho alveolar lavage (BAL) from IL-5 transgenic animals, suggestive of specific binding. Human peripheral blood and BAL eosinophils were shown to interact rapidly with labeled influenza and labeled respiratory syncytial virus (RSV) *in vitro*. At incubation, eosinophils showed subsequent no, diffuse- and clustered binding of virus, and at 2 hours after incubation all eosinophils had bound virus. Labelled RSV co-localized to major basic protein at the eosinophil surface during diffuse viral binding. Later labelled virus appeared to dissociate from MBP and ended up in the lysosomal compartment. Infectivity of epithelial cells by RSV was reduced after RSV was allowed to interact with eosinophils for 2 hours as compared to eosinophils incubated with RSV for 5 minutes only ($p<0.028$). Eosinophils that had taken up virus showed significantly enhanced expression of activation marker CD69 and secretion of IL-8 and IL-6. Other activation markers of eosinophils such as CD11b, CD66b, CD62L and eosinophil cationic protein were not altered, suggestive of mild activation of eosinophils only upon exposure to virus. Interestingly, BAL eosinophils from asthma patients after a rhinovirus-16 challenge, also displayed enhanced CD69 expression, which correlated strongly with asthma complaints (ACQ; spearman R=0.85).

Conclusion: We show that murine and human eosinophils bind, take up and reduce infectivity of respiratory viruses, suggestive of anti-viral properties. Both the presence of MBP on the cell surface and selective and mild activation of eosinophils are indicative of piecemeal degranulation. Together these results suggest a rapid anti-viral role of eosinophils and its coinciding mild activation may contribute to asthma complaints during virus-induced loss of asthma control.

POSTER 53

DIFFERENTIAL ACTIVATION OF AIRWAY EOSINOPHILS PROMOTES IL-13 INDUCED PULMONARY RESPONSES FOLLOWING ALLERGEN PROVOCATION

Elizabeth A. Jacobsen[1], Alfred Doyle[1], Dana Colbert[2], Katie R. Zellner[1], Cheryl A. Protheroe[1], William E. LeSuer[1], Nancy A. Lee[2], and James J. Lee[1]

[1] Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259, [2] Divisions of Hematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

Background: Allergic respiratory inflammation is linked with eosinophil infiltration into the airways and a concurrent increased expression of IL-13. Mice deficient in eosinophils display reduced airway IL-13, suggesting a link between airway eosinophil activities and IL-13 expression. Through the use of eosinophil-deficient mice and adoptive transfer techniques our data suggests that eosinophils modulate the Th2 immune microenvironment through several mechanisms. These studies determine the relative importance of eosinophil activation and induced expression of IL-4 and IL-13 to the induction of allergic Th2 polarized immune and/or inflammatory responses.

Methods: Purified blood-derived wild type or cytokine deficient (IL-4$^{-/-}$ and IL-13$^{-/-}$) eosinophils were either untreated or pre-treated with cytokines prior to adoptive transfer into the lungs of allergen challenged eosinophil-deficient mice (PHIL). Eosinophils were adoptively transferred directly into the lung (i.e., intratracheal instillation (*i.t.*)) into mice each day of allergen challenge using an established acute ovalbumin (OVA) sensitization/challenge protocol. Allergen-induced pulmonary changes in Th2 cytokine levels, histopathologies, and accumulation of leukocyte populations were assessed.

Results: The activation-state of eosinophil functions were characterized by the immune modulating capacities of eosinophils in the allergen provocation models. In particular, GM-CSF treatment primed eosinophils sufficiently to induce their recruitment to the lung draining lymph nodes to promote dendritic cell accumulation and T cell proliferation, yet did not induce pulmonary pathologies. Conversely, treatment of eosinophils with a combination of IL-33, IL-4, and GM-CSF was sufficient to induce pulmonary DC, macrophage, and T cell accumulation as well as induced histopathologies. These allergen-induced Th2 pulmonary pathology responses, in PHIL mice, were dependent on the expression of IL-13 (and not IL-4) by eosinophils.

Conclusion: Our data demonstrates that eosinophils exposed to various cytokines associated with Th2 pulmonary immune responses induce unique immune effector functions. Moreover we demonstrate that activated eosinophils, exposed to GM-CSF and IL-33, promote these allergen-induced Th2 pathologies through the expression of eosinophil-derived IL-13. Thus, the role(s) of eosinophils and other IL-13 producing cells are likely interrelated and amplify the immune responses allergic respiratory inflammation.

This work is supported by Mayo Foundation for Medical Education and Research and grants from National Institutes of Health [NAL (HL058723), JJL (HL065228), and JJL (HL124165)] and American Heart Association [EAJ (11SDG7510043)].

POSTER 54

LOCAL AUTOIMMUNITY IN SEVERE EOSINOPHILIC ASTHMA

Manali Mukherjee, PhD[1], David Bulir BSc[2], Katherine Radford, MSc[1], Chynna Huang, RRT [1] Melanie Kjarsgaard[1] RRT, James J Lee, PhD[3], James Mahony[2] PhD, Parameswaran Nair, MD, PhD[1]

[1]Division of Respirology, McMaster University, Hamilton, Ontario, Canada and [2]Department of Pathology and Molecular Medicine, McMaster University and [3]Division of Pulmonary Medicine, Mayo Clinic, Scottsdale, AZ

Background: Lymphoid aggregates and submucosal B-cells were recently reported in lung biopsies from severe asthmatics (AJRCCM 2012; 186: 501-7). Since, eosinophils are known to support B-cell survival and maturation; we hypothesized that increased presence of immunogenic entities like eosinophil peroxidase (EPX) can trigger *in situ* antibody production by submucosal B cells and thereby, lead to loss of immune tolerance in such airways.

Methods: In an attempt to investigate a plausible autoimmune pathomechanism underlying the severity of eosinophilic asthma (sputum eosinophils >3%), we analysed induced sputum (IS) samples for evidence of anti-EPX antibodies. Total Immunoglobulins (Igs) were immunoprecipitated from the processed IS supernatants using Pierce™ Protein A/G beads (Thermo Scientific). To detect anti-EPX Igs, the eluates were reacted with synthetic human EPX (Lee Biosciences, MO, USA) and mouse anti-EPX antibody (clone MM25-429.1.1), and subsequently probed with rabbit anti-mouse IgG secondary antibody (Abcam) on a 'dot-blot'. For re-validation, an in-house Sandwich ELISA was developed using Biotinylated mouse anti-EPX monoclonal antibody (clone MM25-82.2.1, 1µg/ml) that specifically detected the binding of synthetic EPX to sputum Igs, coated on micro-titre plates. As a measure of local B-cell activity, sputum levels of the cytokine B-cell activating factor (BAFF) were analysed using a commercial ELISA kit (R&D Systems). To

further understand, the propensity of severe asthmatics towards developing an autoimmune condition, we used a routine clinical anti-nuclear antibody (ANA) detection kit (Human Diagnostics Worldwide, Germany).

Results: Proportion of patients with anti-EPX Igs was significantly greater (P<0.001, ANOVA) in the eosinophilic asthma group (EA, n = 24, sputum eosinophils ≥ 3%) as compared to the non-eosinophilic asthmatics (NEA, sputum eosinophils <3%), non-eosinophilic, non-asthma control group (n=10) and healthy volunteers (n=8). Remaining IS supernatants (with excess volume) were analysed for their endogenous BAFF content. Significantly increased BAFF levels were detected in the EA sputum samples (125 ± 84.9 pg/ml, n=17) in contrast to the NEA group (28.3 ± 77.9, n = 8), while undetectable in both healthy and non-asthma controls (p<0.001, ANOVA). In addition, the BAFF levels positively correlated with the detected anti-EPX blot densities (r= 0.5496, P =0.0054). Further on, significant positive correlations were documented for anti-EPX Ig levels and eosinophil percentage (r=0.529, p=0.0001), sputum EPX (r=0.736, p<0.0001) and daily inhaled corticosteroid dose (r=0.5385, p=0.0018). 18 out of 24 EA patients with evidence of anti-EPX Igs were maintained on daily prednisone (median-dose 10mg). Furthermore, 6 out of 8 prednisone-dependent EA patients with detectable anti-EPX Igs were positive for circulating ANAs.

Conclusion: We hereby report a previously unrecognized autoimmune component in severe eosinophilic asthma associated with increased B-cell activity in the airways and the requirement for increased corticosteroid maintenance therapy.

Grant support: PN is supported by Canada Research Chair Program

POSTER 55

NOVEL SCORING SYSTEM AND ALGORITHM FOR CLASSIFYING EOSINOPHILIC CHRONIC RHINOSINUSITIS: THE JESREC STUDY

Takahiro Tokunaga[1], Masafumi Sakashita[1], Takahiro Ninomiya[1], Yoshimasa Imoto[1, 2], Taiyo Morikawa[1], Kaori Tomita[1], Tetsuji Takabayashi[1], Shigeharu Fujieda[1] & JESREC group

[1] Department of Otorhinolaryngology Head & Neck Surgery, University of Fukui, Fukui, Japan, [2] Division of Allergy and Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago

Background: In this study, we wanted to set objective clinical criteria for the diagnosis of refractory eosinophilic chronic rhinosinusitis (ECRS) before surgery.

Methods: This was a retrospective study conducted by 15 institutions participating in the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC). We analyzed 1716 patients treated with ESS who did not receive systemic/topical corticosteroids. In order to investigate the relation between eosinophil infiltration in tissue and recurrence of CRS, in all cases the number of infiltrated eosinophils in the submucosa of the ethmoid cavity or in nasal polyps were counted under the microscope. Risk of recurrence was estimated using Cox proportional hazard models. Multiple logistic regression models and receiver operating characteristics curves were constructed to create the diagnostic criterion for ECRS.

Results: This analysis showed that the cutoff value of 70/HPF of the number of infiltrated eosinophils in nasal polyps presented the most significant difference in recurrence of CRS (P < 0.001). Cases that presented number of eosinophils in nasal polyps equal or higher than 70/HPF were defined as ECRS. According to this definition, 672 out of the 1716 patients (39.2%) had ECRS, and 1044 (60.8%) had non-ECRS. To diagnose ECRS, the JESREC scoring system assessed bilateral disease (3 points), presence of nasal polyps (2 points), blood eosinophilia (2-5% for 4 points, 5-10% for 8 points, >10% for 10 points), and dominant shadow of ethmoid sinuses in computed tomography (CT) scans (2 points). If the total points of the patient are 11 or more, we could diagnose ECRS (sensitivity: 83%, specificity: 66%). Blood eosinophilia (>5%), ethmoid sinus disease detected by CT scan, bronchial asthma, aspirin and non-steroidal anti-inflammatory drugs intolerance were associated significantly with recurrence. The diagnostic algorithm for refractory ECRS was created based on all the results.

Conclusion: We subdivided CRSwNP in non-ECRS, mild, moderate, and severe ECRS according to our algorithm. This classification was significantly correlated with prognosis, and could be useful for the explanation of CRS to patients.

POSTER 56

EOSINOPHIL DEGRANULATION AND THE RELEASE OF EOSINOPHIL PEROXIDASE CONTRIBUTES TO THE INDUCED INFLAMMATION OCCURRING IN MICE FOLLOWING SKIN EXPOSURE TO TMA

Huijun. Luo[1], Cheryl A. Protheroe[1], Emily D. Blum[2], Allison D. Fryer[2,3], Nancy A. Lee[4], D. B. Jacoby[2,3], and James J. Lee[1]

[1]Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259, [2]Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR 97239, [3]Department of Physiology & Phar-

macology, Oregon Health & Science University, Portland, OR 97239, [4]Divisions of Hematology/Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, AZ 85259

Background: Contact reactions to allergens and non-classical allergen toxicants such as trimellitic anhydride (TMA) are accompanied by the specific accumulation of eosinophils (and associated eosinophil degranulation), localized skin inflammation, and concomitant increases in site-specific itch responses. We have recently shown that TMA-induced skin inflammation as well as increases in dermal sensory innervation and the concomitant increase in itching were each eosinophil-dependent events. This demonstrates that a necessary link exists between eosinophils and allergen-induced skin inflammation but does not implicate responsible mechanisms.

Methods: Wild type (BALB/c), eosinophil deficient PHIL, and eosinophil peroxidase (EPX) as well as major basic protein (MBP-1) knockout mice were used in a TMA contact hypersensitivity model. High definition videography was used to record the extent of induced itching, prior to measuring local inflammation and the recovery of tissue biopsies for gene expression, histology, immunohistochemistry staining, and imaging of skin innervation.

Results: EPX knockout mice displayed decreases the skin inflammation (i.e., decreases in eosinophil accumulation, mast cell activation, and skin thickness) that were similar to the reduced inflammatory events (relative to wild type controls) occurring in eosinophil deficient PHIL mice. More importantly, these mice also displayed decreases in itching. Significantly, MBP-1 knockout mice showed no difference in any of these parameters compared with wild type mice, demonstrating differential effector functions mediated by the release of specific secondary granule proteins. Follow-up studies with agents directed against the activities mediated by eosinophil peroxidase (e.g., administration of either anti-EPX monoclonal antibodies or the peroxidase-targeting drug Resorcinol) suggest that directly targeting EPX may represent an underappreciated therapeutic approach to eosinophilic skin diseases.

Conclusions: The release of EPX (but not MBP-1) by skin eosinophils appears to be a causative mechanism contributing to the inflammation and itching that occurs following exposure to the non-classical allergen TMA.

This work is supported by Mayo Foundation for Medical Education and Research and grants from National Institutes of Health [NAL (HL058723), JJL (HL065228), and JJL and DBJ (AR061567)].

POSTER 57

INNATE IMMUNE SYSTEM CROSSTALK: EOSINOPHILS MEDIATE IMMUNE MODULATION OF DENDRITIC CELLS

Fabiola Cortinas-Elizondo[1], Kayluz Frias Boligan[1], Kathrin Kühni-Boghenbor[2], Michael Stoffel[2], Hans-Uwe Simon[1] and Stephan von Gunten[1]

[1]Institute of Pharmacology, University of Bern. Bern, Switzerland, [2]Division of Veterinary Anatomy, University of Bern, Bern, Switzerland

Background: Eosinophil granulocyte infiltration is a predominant feature of allergic immune responses. However, despite being a significant player in these diseases, its exact function in the pathogenesis and interaction with other immune cells remains to be explored.

Methods: We performed functional assays on monocyte-derived dendritic cells (DCs) upon coculture with eosinophils. Upregulation of several maturation markers were analyzed by flow cytometry. Functional activation of DCs was tested in mixed leukocyte reactions: T-cell proliferation was measured via ^{3}H-Thymidine incorporation assay and cytokine responses in cell supernatants and intracellularly. Chemotaxis assays, live-cell imaging, as well as confocal and scanning electron microscopy were also used to elucidate the crosstalk between eosinophils and DCs.

Results: Our results show that eosinophils are capable of activating DCs in vitro as seen by the upregulation of MHC II, several costimulatory molecules and cytokine secretion. Additionally, this activation requires a physical crosstalk between these two cells that might involve the formation of an immune synapse.

Conclusions: Our results reveal a novel role of eosinophils in the activation of DCs to orchestrate allergic immune responses, which besides the new mechanistic insights, disclose potential novel treatment approaches for allergic or hypereosinophil chronic inflammatory diseases.

POSTER 58

TIM-4 EXPRESSED ON EOSINOPHILS FROM LUNG- AND GUT-DRAINING LYMPH NODES COSTIMULATES T CELL EXPANSION

Haibin Wang[1], Lisa A, Spencer[1] and Peter F. Weller[1]

[1]Divisions of Infectious Diseases and Allergy and Immunology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Bachground: TIM-4, T cell immunoglobulin and mucin domain (TIM)-4, is exclusively expressed on antigen-presenting cells (APCs), while its counter ligand TIM-1 is expressed on activated T cells. Eosinophils are emerging as professional APCs, but the expression of TIM-4 by eosinophils has not been defined.

Methods: Expressions of TIM-4 on eosinophils from different lymphoid organs of IL-5 transgenic mice were examined by flow cytometry. TIM-4 positive or negative eosinophils were incubated with CFSE-labeled T cells in the presence of plate-coated anti-CD3 and soluble anti-CD28 for 3 days with or without various doses of anti-TIM-4 blocking antibody, and T cell proliferation was assessed by flow cytometry.

Results: We found that TIM-4 was expressed on murine eosinophils in the lung- and gut-draining lymph nodes, but not in the spleens of IL-5 transgenic mice. TIM-4$^+$ eosinophils, purified from lung- and gut-draining lymph nodes, costimulated and increased T cell expansion induced by anti-CD3 and anti-CD28 Abs, compared to TIM-4$^-$ eosinophils purified from spleens. TIM-4$^+$ eosinophil costimulation function was blocked by anti-TIM-4 antibody in a dose-dependent manner.

Conclusions: TIM-4 is selectively expressed by eosinophils from lung and gut-draining lymph nodes, and can function as a costimulatory molecule to enhance T-cell expansion, suggesting that eosinophils acquired more capabilities as immune regulatory cells after migrating into lymph nodes.

Grant support: This study was funded by NIH grants R37-AI020241 and R01-AI051645 to Peter F. Weller, M.D., and NIH R01-HL095699 to Lisa A. Spencer, Ph.D.

POSTER 59

ALLERGEN-INDUCED INCREASES IN IL-25 AND IL-25 RECEPTOR EXPRESSION IN MILD ASTHMATICS: MECHANISM FOR PROMOTING LUNG HOMING OF MATURE AND LINEAGE-COMMITTED PROGENITORS OF EOSINOPHILS

W Tang[1,2], SG Smith[1], JP Oliveria[1], BM Watson[1], GM Gauvreau[1], PM O'Byrne[1] and R Sehmi[1].

[1]Division of Respirology, Department of Medicine, McMaster University, [2]Department of Respirology, Ruijin Hospital, Shanghai, China.

Background: IL-25 (IL-17E) is a pro-inflammatory mediator that belongs to the IL-17 cytokine family and plays a pivotal role in the maintenance of type 2 immune responses. In mouse studies, administration of IL-25 in the lungs causes eosinophilic inflammation and airway hyperresponsiveness (AHR) and inhibition of IL-25 prevents ovalbumin-induced AHR. Although an important role for IL-25 has been established in animal models of allergic asthma, the kinetics of IL-25 and IL-25 receptor (IL-17RA and IL-17RB) expression in innate and adaptive immune cells during allergic inflammatory responses in human asthmatics has not been investigated. We have recently reported that the expression of IL-17RA and IL-17RB on eosinophils and plasma level of IL-25 are significantly greater in allergic asthmatics compared with atopic non-asthmatics and non-atopic normal subjects. In this study, we examined the kinetics of IL-25 and receptor fluctuation on mature and immature eosinophils and Th2 memory cells in the allergic asthma following allergen inhalation challenge. We have investigated a functional role of IL-25 on eosinophil biological responses.

Methods: In a diluent controlled allergen-inhalation study 14 subjects with mild asthmatics who developed allergen-induced early and late bronchoconstrictor responses were recruited. All subjects were skin prick test positive, FEV1≥70% predicted, >12% reversibility of FEV1, PC20≤16 mg/ml, and were steroid-naïve with infrequent use of inhaled ß2-agonists. Blood samples were collected at baseline, 7h and 24h following allergen- or diluent-inhalation challenge. Surface expression of IL-17RA, IL-17RB and intracellular IL-25 were evaluated by flow cytometric analyses of mature eosinophils (CD45+16- cells), eosinophil-lineage committed progenitor cells (EoP; CD34+45+125+) and Th2 memory cells (CD3+CD4+CRTH2+). Plasma levels of IL-25 were measured by ELISA and cell migration studies were performed using microboyden chambers for mature eosinophils and transwell chambers for eosinophil progenitor cells.

Results: There was a significant increase in the numbers of mature eosinophils and EoP, but not Th2 memory cells, expressing components of IL-25 receptor at 7 h and 24 h post-allergen but not diluent challenge. Plasma levels of IL-25 increased post-allergen challenge and a significant increase in intracellular IL-25 levels was detected in eosinophils post-allergen. IL-25 had no direct effect on migrational responses of mature eosinophils and EoP but pre-exposure to IL-25 (optimal at 1 pg/ml), *in vitro,* primed the

subsequent migrational of these cells to Eotaxin (10 nM) and SDF-1 (10 ng/ml) respectively. This priming effect was inhibited in the presence of an IL-25 neutralizing antibody.

Conclusions: The findings of this study suggest that increased production of IL-25 in allergic asthmatic responses may play a role in promoting the development of airway eosinophilia by priming the migrational responsiveness of mature and immature eosinophils.

Grant support: Dr. SG Smith holds a post-doctoral fellowship award from the Father Sean O'Sullivan Research Centre, Hamilton.

POSTER 60

PREDICTING BLADDER CANCER PATIENT RESPONSE TO BACILLUS CALMETTE-GUÉRIN USING A NOVEL DIAGNOSTIC STRATEGY ASSESSING THE PRE-TREATMENT TUMOR IMMUNE MICROENVIRONMENT

Rafael Nunez-Nateras[1], Cheryl A. Protheroe[2], Melissa L. Stanton[3], Sergei I. Ochkur[2], Elizabeth A. Jacobsen[2], Yue-Xian Hou[1], Erin Ferrigni[1], Paul E. Andrews[1], Thomas V. Colby[3], Nancy A. Lee[2], Erik P. Castle[1], and James J. Lee[2]

[1]Department of Urology, Mayo Clinic Arizona, Phoenix, Arizona, USA, [2]Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, Arizona, USA, [3]Department of Laboratory Medicine/Pathology, Mayo Clinic Arizona, Scottsdale, Arizona, USA

Background: Standard-of-care treatment for non-muscle invasive bladder cancers is intravesical administration of live attenuated Bacillus Calmette-Guerin (BCG). However, there is currently no ability at the time of initial diagnosis to identify which patient will respond to this immune adjuvant treatment. As a consequence, bladder cancer patients are generally treated using a "one size fits all" approach with only about 60% of treated patients responding to BCG. Currently there is no ability at the time of initial diagnosis to identify patients responsive to this standard-of-care treatment.

Methods: Bladder cancer patients presenting the carcinoma *in situ* (Tis) tumor subtype were stratified on the basis of their subsequent responsiveness to treatment with BCG. A total of 38 patients met inclusion criteria (20 patients responsive to BCG and 18 unresponsive subjects). Control bladder samples were derived from cold autopsy biopsies. Immunohistochemical assessments of bladder biopsies were used to quantify eosinophil infiltration/degranulation and the number of tumor infiltrating Th2 polarized vs. Th1 polarized T lymphocytes.

Results and Limitations: Subjects without urogenital disease displayed a nominal eosinophil infiltrate with no evidence of degranulation or infiltrating lymphocytes. In contrast, Tis bladder tumors often displayed a robust eosinophilia accompanied by degranulation and >3-fold more infiltrating GATA-3$^+$ relative to T-bet$^+$ T lymphocytes (G/T). In a retrospective study of bladder cancer patients, these independent measures each had statistically significant value to discriminate between responsive and unresponsive patients following BCG induction. In addition, the data show that eosinophil numbers and degranulation as well as assessments of tumor G/T levels were dramatically decreased in response to BCG treatment. More importantly, an algorithm integrating these metrics (Th2 Signature Biomarker) provided an even greater diagnostic tool capable of stratifying patients prior to BCG treatment.

Conclusions: Bladder tumor immune microenvironments lacking Th2 polarization were prevailing indicators of how patients responded to BCG therapy. Integration of multiple measures of tumor immune polarization that include measure for the presence of eosinophils and degranulation represents a clinically relevant pre-treatment screening strategy of non-muscle invasive bladder cancer patients.

This work is supported by Mayo Foundation and a grant from the NIH/NCI (JJL: CA112442).

POSTER 61

A PROOF-OF-CONCEPT STUDY WITH A NOVEL ANTI-IL-13 MONOCLONAL ANTIBODY (RPC4046) IN ADULTS WITH EOSINOPHILIC ESOPHAGITIS: DESIGN AND OUTCOME MEASURES OF THE HEROES STUDY

Ikuo Hirano[1], Sandeep K. Gupta[2], Michael Grimm[3], Sheila Gujrathi[3], Alain M. Schoepfer[4], Alex Straumann[5], Heather Smith[3], Amy Woo[3], Gregg Timony[3], Robert Peach[3], Paul Frohna[3], Steven J Ackerman[6], Evan S. Dellon[7]

[1] Northwestern University Feinberg School of Medicine, Chicago, Illinois USA, [2] Indiana University Department of Pediatrics, Indianapolis, Indiana, USA, [3] Receptos, Inc., San Diego, California USA, [4] University Hospital Lausanne, Lausanne Switzerland, [5] Swiss EoE Research Network, Olten, Switzerland, [6] University of Illinois, Chicago, Illinois USA, [7] University of North Carolina, Chapel Hill, North Carolina USA

Introduction. Eosinophilic esophagitis (EoE) is a chronic, immune/food antigen-mediated disease characterized clinically by esophageal dysfunction and histologically by eosinophil-predominant inflammation with overexpression of cytokines, including

interleukin 13 (IL-13). RPC4046 is a humanized, high-affinity, neutralizing, monoclonal antibody that prevents IL-13 binding to both IL-13Rα1 and IL-13Rα2, which is in clinical development for EoE.

Design. The HEROES study is an ongoing, randomized, double-blind, placebo-controlled, proof-of-concept study (NCT02098473) in approximately 90 adults with symptomatic EoE who are being randomized in a 1:1:1 ratio to receive one of two doses of RPC4046 (180 or 360 mg SC) or matching placebo weekly for 16 weeks. Eligibility requires ≥15 eosinophils per high power field at two esophageal levels, plus at least 4 days with symptoms of dysphagia during a 2-week evaluation period during screening. The primary objective of the trial is to characterize the effect of RPC4046 on eosinophil counts in esophageal biopsy samples from subjects with symptomatic EoE. The secondary objectives are to characterize the effects of RPC4046 on clinical symptoms and endoscopic score, and to characterize the safety and tolerability of RPC4046. A safety monitoring committee is overseeing the study.

Outcome Measures. Change from baseline for mean eosinophil counts from esophageal biopsies is the primary endpoint. For secondary outcomes, EoE symptoms will be evaluated with two EoE-specific patient reported outcome (PRO) instruments, a daily symptom diary and the Eosinophilic Esophagitis Activity Index (EEsAI, Schoepfer 2014) to assess dysphagia daily and over a 7-day recall period, respectively. Circulating biomarkers will include eotaxin-3, periostin, and other markers of eosinophilic inflammation. To characterize the effects of RPC4046 on tissue-level EoE disease pathology, esophageal biopsies will be analyzed histopathologically for epithelial mesenchymal transition (EMT) (Kagalwalla et al 2012), a likely contributor to subepithelial fibrosis in EoE, and by gene expression analysis using a previously described molecular EoE diagnostic panel (Wen et al, 2013). Finally, the distensibility of the esophagus will be measured using the Endolumenal Functional Lumen Imaging Probe (EndoFLIP®, CROSPON, Carlsbad, CA, KWIATEK 2011) Imaging System, which serves as an objective functional endpoint that may be particularly useful when combined with the PRO results.

Conclusion. Subject accrual is currently underway. The design of the HEROES study should provide important safety, clinical, histologic, pharmacodynamic and functional data on the effects and tolerability of RPC4046 in patients with EoE that will assist with design of future clinical trials.

Grant support: Drs. Hirano, Schoepfer, Straumann, and Dellon are clinical investigators participating in this study and are receiving grant support. Drs. Gupta and Ackerman are study advisors.

POSTER 62

A MULTIPLE-FOOD ELIMINATION DIET IS EFFECTIVE FOR THE TEATMENT OF EOSINOPHILIC GASTROENTERITIS

Yoshiyuki Yamada[1], Yuka Isoda[2], Akira Nishi[3], Yuko Jinbo[2], Masahiko Kato[1, 4]

Divisions of [1]Allergy and Immunology, [2]Nutrition and Cooking and [3]Surgery, Gunma Children's Medical Center, Shibukawa, Gunma, Japan, [4]Department of Pediatrics, Tokai University School of Medicine, Isehara, Kanagawa, Japan

Background: Recently, an empiric diet preferentially devoid of the six most common food allergens, milk, soy, egg, wheat, peanuts/tree nuts, and shellfish/fish (6-FED) has been effectively used for the treatment of eosinophilic esophagitis. We employed a multiple-food elimination diet (MFED), which is a modified 6-FED, and analyzed its effectiveness for the treatment of eosinophilic gastroenteritis (EGE) in our hospital.

Methods: The study included four patients with EGE who were diagnosed based on gastrointestinal symptoms and eosinophil infiltration of the gastrointestinal mucosa with ≥20 eosinophils/high-power field. The patients were treated a total of five times with a MFED in our hospital between 2010 and 2014. The patients' clinical data, including imaging and histological findings, and eosinophil, albumin, immunoglobulin G (IgG), and hemoglobin levels, were retrospectively reviewed. The data were compared between before and after the MFED, and the worst and best data before and after the MFED were used for analysis.

Results: One patient was being treated with prednisolone before the MFED. Before the MFED, a low albumin level was observed in one patient, and another patient had a low hemoglobin level. All patients, except the patient who was being treated with prednisolone, had a low serum IgG level. Additionally, all patients showed clinical improvement, and imaging or histological findings improved in three of the four patients after the MFED. Notably, the MFED decreased the eosinophil level and increased the albumin, IgG, and hemoglobin levels in three patients. Interestingly, the MFED resulted in the tapering and subsequent discontinuation of corticosteroid in the patient who was being treated with prednisolone.

Conclusion: These findings suggest that the MFED can improve the clinical findings and laboratory data in patients with EGE. Therefore, the MFED has been chosen as an alternative treatment approach for patients with EGE in our hospital.

Grant support: This project was supported by Research on Intractable Diseases, Health and Labour Sciences research grants from the Ministry of Health, Labour and Welfare of Japan and by a Grant-in-Aid for Scientific Research C from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

POSTER 63

A CURIOUS CASE OF CYCLIC VOMITING WITH EOSINOPHILIA

Narender Vadlamudi, MD, Santhosh Kumar, MD. Virgnia Commonwealth University Medical Center, Richmond, VA

Background: We report a very interesting case of cyclic vomiting with eosinophilia

Case Report: This is case report of a now 12 year old female who developed cyclic vomiting with corresponding raise in peripheral blood eosinophil counts. Endoscopy and colonoscopy was consistent with eosinophilic infiltration and bowel edema of her bowels except the esophagus. Patient also has a positive T-cell clonality. She was successfully treated with bursts of steroids during episodes and now on a trial of Cyclosporine as a steroid sparing agent.

Discussion: This is the first case report of cyclic vomiting with eosinophilia likely due to angioedema limited to the bowels. The pattern of her waxing and waning eosinophil counts with symptoms indicate causality. She has responded very well to oral steroids and with the recent identification of T-cell clone, cyclosporine was started with the hope of limiting her steroid use. Our case demonstrates a methodical work up of a patient with cyclic vomiting and peripheral eosinophilia.

POSTER 64

IMPACT OF TREATMENT ON THE ESOPHAGEAL MICROBIOTA AND BACTERIAL RECEPTOR EXPRESSION IN EOSINOPHILIC ESOPHAGITIS

Sophie A. Fillon[1,2,3,4], J. Kirk Harris[2,5], Lauren N. Henry[1,2,3,4], Ha Na Choe[1,2,3,4], Brandie D. Wagner[5,6], Wendy Moore[1,2,3,4], Dresden Whitehead[1,2,3,4], Katie Amsden[7], Amir F. Kagalwalla[7], Angelika Zalewski[8], Ikuo Hirano[8], Nirmala Gonsalves[8], Mark J. Stevens[2,5], Kathryn A. Biette[1,2,3,4], Joanne C. Masterson[1,2,3,4], Steven J. Ackerman[9], Glenn T. Furuta[1,2,3,4].

[1]Digestive Health Institute, Section of Pediatric Gastroenterology, Hepatology and Nutrition, Children's Hospital Colorado, [2]University of Colorado School of Medicine, Aurora, CO, USA; [3]Gastrointestinal Eosinophilic Diseases Program, [4]Mucosal Inflammation Program; [5]Division of Pulmonology; [6]Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado, Aurora, CO, USA; [7]Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Ann & Robert H. Lurie Children's Hospital of Chicago and Northwestern University, Chicago, IL, USA; Department of Pediatrics, John H. Stroger Hospital of Cook County, Chicago, IL, USA; [8]Department of Medicine, Division of Gastroenterology & Hepatology, Northwestern University, Feinberg School of Medicine, [9]Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, IL, USA

Background: Eosinophilic Esophagitis (EoE) is an allergic disease characterized by upper intestinal symptoms found in association with chronic inflammation and eosinophil infiltration. Few studies have determined the esophageal microbiota in health or disease and those have utilized mucosal biopsies. We have previously reported that the minimally invasive Esophageal String Test (EST), a swallowed Enterotest™ capsule containing a nylon string, can be used to sample the esophageal microbiome in a manner comparable to esophageal biopsies.

Objectives: The overall goal of this study was to identify the esophageal microbiota in EoE and determine whether treatment (proton pump inhibitor, steroid or diet) changes this profile. We hypothesized that clinically relevant alterations in bacterial populations are present with esophageal inflammation in EoE. The approach was to identify the esophageal microbiota in well-defined patients with EoE and determine the impact of treatment on the microbiota and bacterial receptors. We hypothesize that the microbiota varies depending on the state of esophageal inflammation.

Methods: In this prospective study, ESTs were collected from children and adults with EoE, and normal mucosa. Bacterial communities were determined by 16S rRNA gene amplification, 454 pyrosequencing and MiSeq, and compared between health, disease and treatment. ESTs were collected from EoE (n=49) and controls (n=43), half the subjects in both groups were treated with proton pump inhibitor (PPI). All subjects had upper intestinal symptoms with further diagnostic criteria including EoE $\geq$ 15 eos / high power field (HPF) and other causes excluded; and normal esophageal mucosa. Sequencing results were analyzed by subject group. To determine the impact of treatment on bacterial receptor expression, human esophageal epithelial cells (EPC2-hTERT) were stimulated with the proton pump inhibitor Omeprazole (50μM) or Fluticasone propionate (1μM) in the presence of a Th2 (IL-13 and IL-5) and Gram - (LPS) micromilieu. Bacterial receptor expression was determined by western blot.

Results: We previously reported that the relative abundance of *Haemophilus* was significantly increased in untreated EoE subjects compared with normal subjects. *Haemophilus* was present in all subjects independent of diagnosis; however subjects on PPI (n=42) had a 50% increase in *Haemophilus* relative abundance compared to untreated subjects (n=15) $p<0.01$. *Haemophilus* relative abundance was slightly decreased by 25% in subjects treated with steroids (n=23) compared to untreated (n=15). No significant differences in bacterial genera (with a relative abundance >1%) were observed between the microbiota on biopsies and matching ESTs

in EoE subjects (n=7). Expression of the bacterial receptor for *Haemophilus,* ICAM-1, was increased by PPI treatment and decreased by steroid treatment in esophageal epithelial cells.

Conclusions: The microbiota is altered in EoE from that found in the normal mucosa. Moreover, the bacterial composition and bacterial receptor expression in the esophageal epithelium is altered by treatment.

Grant support: American Partnership For Eosinophilic Disorders (APFED) HOPE Grant and KL2 grant from the Colorado CTSI (KL2 TR001080 (SF), NIH/NCRR Colorado CTSI Grant Number UL1 RR025780 and NIH DK100303 (GTF), Campaign Urging Research for Eosinophilic Diseases (CURED) (SJA/GTF), Ann & Robert H. Lurie Children's Hospital of Chicago CRU Grant 1UL1RR025741, and Buckeye Foundation (AFK).

POSTER 65

DEXPRAMIPEXOLE FOR THE TREATMENT OF CHRONIC RHINOSINUSITIS WITH NASAL POLYPS: PRELIMINARY FINDINGS IN AN OPEN-LABEL PROOF OF CONCEPT TRIAL

Michael E. Bozik[1], Gregory T. Hebrank[1], Stella Lee[2], Berrylin J. Ferguson[2], James Mather[1], Mary S. Sullivan[1], Donald Archibald[1], Reynold A. Panettieri[3], Steven I. Dworetzky[1]. [1]Knopp Biosciences LLC, [2]UPMC Department of Otolaryngology, [3]University of Pennsylvania

In our previous clinical experience in over 1000 ALS subjects without eosinophilia, dexpramipexole demonstrated pronounced dose- and time-dependent eosinophil- and basophil-lowering activity. This effect developed after 1 month on treatment, required 3-4 months to reach its maximum, and remained constant throughout treatment. In our ongoing Phase 2, open-label, multi-center proof-of-concept study, the primary objectives are to evaluate the clinical effects of oral administration of dexpramipexole in reducing the number of eosinophils in the peripheral blood and in improving nasal polyp score when administered to subjects with chronic rhinosinusitis with nasal polyps (CRSwNP) and eosinophilia. Approximately 20 subjects are enrolling across 2-4 study centers. Following the screening period, eligible subjects receive oral dexpramipexole 150 mg BID for 6 months (with an optional 6-month extension phase). To be eligible, subjects require a confirmed diagnosis of CRSwNP, defined by the presence of at least two of the following symptoms prior to enrollment: anterior and/or posterior mucopurulent drainage, sinonasal obstruction, hyposmia, and endoscopic documentation of bilateral polyps in the middle meatus, or imaging by CT with confirmation of bilateral mucosal disease. In addition, eligible subjects require a documented peripheral absolute eosinophil count >300 cells/µL during the screening period and a bilateral total polyp score of ≥4 (with a unilateral score of ≥2 for each nostril). A total of 7 subjects have been enrolled, of which 3 have passed the Month 3 timepoint.To date, 6/7 subjects have shown absolute eosinophils counts significantly and persistently reduced from baseline values with the majority of subjects reaching a maximum effect by Month 2. By Month 1, one subject had a marked reduction from a baseline eosinophil count of $1.18x10^3$/µl to $0.03x10^3$/µl correlated improvements in polyp score, olfaction and FEV1. At Month 2, five subjects had absolute eosinophil counts ≤$0.01x10^3$/µl, which is a larger magnitude of response observed previously in the ALS subjects. Similar to our previous clinical experience, dexpramipexole at the 150 mg BID dose remains well tolerated with no drug-related serious adverse events reported to date. Overall, the preliminary study results are encouraging in demonstrating significant dexpramipexole eosinophil-lowering effects in subjects with eosinophilia, and with a faster onset of action and a larger magnitude of effect in CRS-NP subjects than was seen in ALS subjects. Updated clinical results will be presented at the conference.

POSTER 66

SOURCE OF INTERLEUKIN-5 IN FAMILIAL EOSINOPHILIA

Senbagavalli Prakash[1], Thomas B. Nutman[1], Calman Prussin[2], Yuzhi Yin[2], Tamika Magee[3], Amy D. Klion[1]

[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, [2]Mast Cell Biology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, [3]Clinical Parasitology Section, Laboratory of Parasitic Diseases, Clinical Research Directorate/CMRP, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

Background: We have previously described a large kindred with autosomal dominant transmission of marked eosinophilia (>1,500/µL). Whereas most affected family members remain asymptomatic with a normal life span, progression to hypereosinophilic syndrome has occurred in 3 family members. Preliminary studies revealed no evidence of an intrinsic abnormality of eosinophil development, activation or apoptosis. Though the gene has been mapped to an 18 cM portion of chromosome 5q31-33 (LOD 6.49) that contains many of the cytokine genes implicated in eosinophilia, candidate gene and whole genome sequencing have been unsuccessful to date in identifying the causative mutation. Analysis of peripheral blood mononuclear cells (PBMC) microarray data

revealed marked upregulation of IL-5 expression in affected family members. The aim of the present study is to determine the cellular source of this increased IL-5 mRNA expression and to determine whether it is associated with production of IL-5 protein.

Methods: PBMC from affected and unaffected family members were isolated using density gradient centrifugation and viably frozen. For some experiments, thawed PBMC were separated by surface phenotype using magnetic bead separation or flow sorting prior to use. RNA was isolated using standard methods to assess IL-5 expression using Taqman PCR. Ct values >35 cycles were considered undetectable. For assessment of IL-5 protein, PBMCs were cultured with and without PMA for 18h prior to intracellular staining for flow cytometric analysis of cytokine production. Supernatants were collected from parallel cultures for cytokine measurement by suspension array.

Results: IL-5 mRNA was detected at increased levels in PBMC from all 10 affected family members (1/ΔCt: 0.05-0.11) as compared to 3 of 9 unaffected family members (1/ΔCt: 0.04, $p<0.01$, Fisher's exact test). To begin to assess the source of this message, PBMC from 4 affected and 4 unaffected family members were separated using magnetic beads into CD3+ and CD3- subsets. IL-5 mRNA was detected in both CD3+ and CD3- cells in affected, but not unaffected, family members. Further, magnetic bead cell separations using PBMC from 6 affected and 6 unaffected family members demonstrated that IL-5 mRNA was detectable in CD4+ cells and CD19+ cells in affected, but not unaffected, family members. Using cells sorted by flow cytometry, IL-5 mRNA was detected not only in CD4+ and CD19+ cells, but also in CD14+ and CD8+ cells from 5 affected family members. Despite multiple attempts, intracellular IL-5 has not been detected by flow cytometry in unstimulated fresh or frozen PBMC from affected family members and IL-5 levels in CD3+CD4+ lymphocytes stimulated with SEB or PMA have been in the normal range. In preliminary experiments using PBMC from 2 affected family members and 2 controls, increased levels of IL5, IL-4, IL-13 and GM-CSF were detected in PMA-stimulated PBMC supernatants from affected family members as compared to controls.

Conclusion: Despite upregulation of IL-5 mRNA expression in affected family members in multiple cell types, production of IL-5 protein has not been confirmed by flow cytometry. Preliminary results demonstrating increased IL-5 production by PMA-stimulated PBMC from affected family members could be consistent with an alternate cellular source of IL-5 protein (such as ILCs) and/or a small, but universal, increase in IL-5 expression by PBMCs.

Grant funding: This study was funded by the Division of Intramural Research, NIAID, NIH. Funded by the NCI Contract No. HHSN261200800001E. This research was supported in part by the National Institute of Allergy and Infectious Diseases.

POSTER 67

EOSINOPHILS REGULATE INTESTINAL B- AND T-CELL RESPONSES FOLLOWING INFECTION WITH THE NEMATODE *HELIGMOSOMOIDES POLYGYRUS*

Julia Strandmark[1], Sebastian Rausch[1], Claudia Berek[2], Susanne Hartmann[1].

[1]Freie Universität Berlin, Dept. of Veterinary Medicine, Institute of Immunology Robert-von-Ostertagstr. 7-13, 14163, Berlin, Germany, [2]Deutsches Rheuma-Forschungszentrum (DRFZ) Berlin, Charitéplatz 1, 10117, Berlin, Germany

Background: Acute infection with the intestinal nematode *Heligmosomoides polygyrus* results in a strong Th2 response, expansion of IgG1 antibodies and eosinophilia. Eosinophils have recently been shown to play a role in plasma cell maintenance in the bone marrow, as well as in IgA class switching in the gut associated lymphoid tissue (GALT), but the role of eosinophils in antibody production in an infectious setting remains unclear.

Methods: ΔdblGATA-1 and BALB/c wild type mice were infected with 200L3 *H. polygyrus* larvae. Serum antibody titres were measured by ELISA and Flow cytometry was used to assess germinal centre reactions, the relative abundance of $IgG1^+$ B-cells and Th2 induction, 14 days post infection.

Results: Eosinophil deficiency leads to a significant augmentation in the rapid expansion of serum IgG1 antibodies seen in acute *H. polygyrus* infection. Similarly, $IgG1^+$ B-cells are substantially elevated in the Peyer's patches, spleen and draining lymph nodes of ΔdblGATA-1 mice as compared to BALB/c. Furthermore, local Th2 induction, as measured by GATA-3 expressing $CD4^+$ T-cells and IL-4 secretion, is significantly enhanced in the absence of eosinophils. Finally, although adult parasite burdens are comparable between mouse strains, parasite fecundity, as measured by individual female egg output, is significantly impaired in worms that develop in ΔdblGATA-1 mice.

Conclusion: Taken together these data demonstrate a novel role fore eosinophils during *H. polygyrus* infection, whereby they support parasite reproduction by suppressing initial expansion of IgG1 antibodies and regulating Th2 induction.

POSTER 68

EOSINOPHILS PROTECT ALVEOLAR MACROPHAGES FROM ACUTE RESPIRATORY VIRUS INFECTION *IN VIVO*

Kimberly D. Dyer[1], Martin L. Moore[2], and Helene F. Rosenberg[1]

[1]Inflammation Immunobiology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD USA, [2] Division of Pediatric Infectious Diseases, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA USA

Background: Eosinophils are recruited to the airways as a prominent feature of the asthmatic inflammatory response where they are broadly perceived as promoting pathophysiology. Respiratory viruses are major exacerbants of pre-existing asthma. However, we have recently shown that activated eosinophils have antiviral properties and protect against the negative sequelae of infection with the pneumovirus pathogen, Pneumonia Virus of Mice (PVM; Percopo *et al.* Blood 2013)

Methods: We have generated recombinant PVM strain J3666 which includes the far-red fluorescent protein, mKATE2 (rK2-PVM; Dyer *et al.,* ms. in preparation 2015). We have inoculated wild-type BALB/c and IL-5 transgenic mice (CD2-IL5Tg) with rK2-PVM and performed flow cytometric analysis to identify myeloid cell populations recruited in response to infection. Detection of mKATE2 is used as the surrogate marker for virus infection.

Results: Similar to native PVM strain J3666, recombinant rK2-PVM replicates in lung tissue of wild-type BALB/c mice and generates a lethal infection with an estimated LD50 of 21 TCID50 units/50 µl inoculum. Both the native and the recombinant virus recruit $CD45^+$ leukocytes to the lungs including neutrophils, monocytic macrophages, interstitial macrophages and $CD11b^+$ dendritic cells. Alveolar macrophages (AMs; $CD11c^+$Siglec F^+) are among the most highly susceptible to infection. Here, rK2-PVM was used to localize virus infection in IL-5Tg hypereosinophilic mice. In rK2-PVM infected IL-5tg mice, 44 ± 1% of $CD45^+$ cells are eosinophils (Siglec F^+Gr1^-) versus 7 ± 1% in their wild-type counterparts. Among the most striking findings, we found that 46 ± 3% of AMs from wild-type mice were mKATE+ at day 5 after virus inoculation; in contrast, only 19 ± 1% of AMs from IL-5Tg mice were mKATE+ at the same time point ($p < 0.0001$).

Conclusions: With fluorescent-labeled rK2-PVM, we have tracked infection of myeloid cells in the lungs of wild-type and hypereosinophilic IL-5Tg mice. Consistent with our earlier findings, our results suggest that eosinophils may be antiviral and they may protect cells in the lung from active infection by modulating the activities of AMs. AMs can reduce viral load by phagocytosis of virus-infected cells; however, they can also become infected and can contribute to the pathology through the release of soluble mediators. We are currently generating mice that will permit us to explore the unique contributions of the cytokine IL-5 *vs*. eosinophils. We will also examine the role of virus in promoting eosinophil activation and determine how this may affect alveolar macrophages.

Funding: NIAID DIR AI000943 to HFR.

POSTER 69

EOSINOPHIL AS A UNIVERSAL DETERMINANT UNDERLYING COMMON COMPLEX DISEASES AND QUANTITATIVE INTERMEDIATE TRAITS

Marzyeh Amini MSc[1], Dinara Bashirova MD MSc[1], Judith Vonk PhD[1], Eva Corpeleijn PhD[1], Bram P. Prins MSc[1], LifeLines Cohort Study[2], Marcel Bruinenberg PhD[2], Lude Franke PhD[3], Pim van der Harst MD PhD[4], Salome Scholtens PhD[1,2], Paul de Bakker PhD[5], Gerjan Navis MD PhD[6], Cisca Wijmenga PhD[3], Ronald P Stolk MD PhD[1,2], Bruce HR Wolffenbuttel MD PhD[7], Dirkje S. Postma MD PhD[8], Gerard H. Koppelman MD PhD[9], H Marike Boezen PhD[1], Harold Snieder PhD[1] and Behrooz Z. Alizadeh MD MSc PhD[1]

[1]Department of Epidemiology, University Medical Center Groningen, University of Groningen, The Netherlands; [2]LifeLines Cohort Study, University Medical Center Groningen, University of Groningen, The Netherlands; [3]Department of Genetics, University Medical Center Groningen, University of Groningen, The Netherlands; [4]Department of Cardiology, University Medical Center Groningen, University of Groningen, The Netherlands; [5]Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; [6]Department of Nephrology, University Medical Center Groningen, University of Groningen, The Netherlands; [7]Department of Endocrinology, University Medical Center Groningen, University of Groningen, The Netherlands; [8]Department of Pulmonology, University Medical Center Groningen, University of Groningen, The Netherlands; [9]Department of Pediatric Pulmonology, University Medical Center Groningen, University of Groningen, The Netherlands.

Background: To study the relationship of eosinophil count with twenty complex diseases and quantitative intermediate traits in three classes of metabolic, cardiac, and pulmonary disorders.

Methods: 13,301 unrelated individuals data from LifeLines, a multi-disciplinary large homogenous population-based cohort study in the northern provinces of the Netherlands (N=167,729), were included in this study. Medical and baseline information of the

individuals were collected by self-report and clinical examination. In the metabolic class we focused on body mass index (BMI;kg/m^2), triglycerides (TG;mmol/L), total cholesterol (TC;mmol/L), high density lipoprotein (HDL;mmol/L), low density lipoprotein (LDL;mmol/L), hemoglobin A1c (HbA1c;%), fasting glucose (FG;mmol/L); as well as obesity, metabolic syndrome (MetS), and type 2 diabetes outcomes. The cardiac class comprised systolic blood pressure (SBP;mmHg), diastolic blood pressure (DBP;mmHg), mean arterial pressure (MAP;mmHg), and pulse pressure (PP;mmHg); as well as hypertension and myocardial infarction (MI) outcomes. The pulmonary class included forced expiratory volume in one second (FEV1;L), ratio of FEV1 and forced vital capacity (FEV1/FVC); as well as chronic obstructive pulmonary disease (COPD) and asthma outcomes. The associations were estimated of eosinophil count with intermediate traits and complex diseases by the means of linear and logistic regression, respectively. To address multiple testing, Bonferroni correction was used, which considered $p<8.3\times10^{-3}$ a significant.

Results: 58.2% of population were women (mean age 51.3±11.1). One standard deviation(SD) increase in eosinophil count was significantly associated with increase of 0.04 ($\pm3.5\times10^{-5}$; p<0.0001) SD of lnTG, 0.03 ($\pm1.4\times10^{-5}$; p<0.0001) SD of TC, 0.04 ($\pm2.0\times10^{-5}$; p<0.0001) SD of LDL, and 0.03 ($\pm2.4\times10^{-5}$; p<0.0001) SD of HbA1c; while with decrease of 0.03 ($\pm1.7\times10^{-5}$; p<0.0001) SD of HDL in metabolic class diseases. Eosinophil count was not significantly associated with BMI after correction for multiple testing. In cardiac class diseases, no significant association was found between eosinophil count and blood pressure components. One SD increase in eosinophil count was significantly associated with decrease of 0.05 ($\pm3.4\times10^{-5}$; p<0.0001) SD of FEV1 and 0.09 ($\pm0.5\times10^{-5}$; p<0.0001) SD of FEV1/FVC ratio. A tertile increase of eosinophil count was significantly associated with an increase (OR) 1.14 (95%CI 1.07-1.21; p<0.0001) fold in the risk for obesity, 1.17 (1.10-1.24; p<0.0001) fold in the risk for MetS, 1.22 (1.13-1.31; p<0.0001) fold in the risk for COPD and 1.55 (1.43-1.69; p<0.0001) fold in the risk for asthma.

Conclusions: In a large population based study, we found that eosinophil count was significantly associated with level of TG, TC, HDL, LDL, HbA1c, FEV1, FEV1/FVC, and the risk of obesity, MetS, COPD, and asthma. Our study signifies eosinophil count as a universal risk factor for common metabolic and pulmonary diseases and their associated traits. These findings may ultimately have clinical implication.

Grant support: Mrs. M. Amini was supported by Groningen University Institute for Drug Exploration (GUIDE), UMCG, Groningen, The Netherlands.

POSTER 70

DETECTION OF HUMAN EOSINOPHIL EXTRACELLULAR VESICLES BY NANOSCALE FLOW CYTOMETRY

Ryann Murphy[1], Kelsey Capron[1], John Tigges[2], Vasilis Toxavidis[2], Virginia Camacho[2], Peter F. Weller[1], and Praveen Akuthota[1,3]

[1]Division of Allergy and Inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA USA; [2]Flow Cytometry Core, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA USA; [3]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA USA

Background: Extracellular vesicles (EVs), a group of small membrane-bound structures including exosome and microvesicles, have generated a great deal of recent interest as potential mediators of cell-cell communication. Recently, primary human eosinophils have been demonstrated to secrete exosomes in optimized cell culture conditions.

Objective: Using a novel nanoscale flow cytometry system to detect particles as small as 200 nm in diameter, we sought to identify and characterize EVs released from isolated human eosinophils in cell culture.

Methods: Human eosinophils were purified by negative selection from healthy donors. Eosinophils were incubated for 4 days in 10 ng/ml IL-5 and 1 ng/ml of GM-CSF to allow for EV accumulation in the culture supernatant. Culture medium was depleted of EVs by ultracentrifugation prior to use. Supernatants were depleted of eosinophils and debris with successive centrifugation at 300 x *g*, 5,600 x *g*, and 11,000 x *g*. EVs were then isolated by ultracentrifugation at 100,000 x *g* for 1 hour. Culture medium without cells present subjected to the same protocol served as a negative control. Prior to nanoscale flow cytometry, some samples were incubated with FITC-conjugated anti-CD9 antibody or FITC-conjugated anti-CD63 antibody. Nanoscale flow cytometry was performed using

a Beckman Coulter MoFlo AstriosEQ modified to optimize detection of small particles down to 200 nm in diameter. Electronic noise was gated out during analysis using the signal generated by phosphate buffered saline alone as a reference.

Results: Using nanoscale flow cytometry, isolated EVs from 4 individual healthy donors were detectable from eosinophil culture supernatants. A population of EVs produced by human eosinophils expressed detectable CD9, while CD63 was not consistently detected.

Conclusions: Human eosinophils produce EVs in culture that can be detected by nanoscale flow cytometry.

References: Mazzeo C, Cañas JA, Zafra MP, Marco AR, Fernández-Nieto M, Sanz V, Mittelbrunn M, Izquierdo M, Baixaulli F, Sastre J, Del Pozo V. Exosome secretion by eosinophils: A possible role in asthma pathogenesis. J Allergy Clin Immunol. 2015 Jan 21. [Epub ahead of print]

Grant support: NIH K08 HL116429 (to PA); NIH R37 AI020241 (to PFW)

POSTER 71

EOSINOPHILS PROMOTE ANTIVIRAL HOST DEFENSES AGAINST INFLUENZA A VIRUS INFECTION

Amali E. Samarasinghe,[1,2,3] Rossana C.N. Melo,[4] Susu Duan,[5] Kim S. LeMessurier,[1,2] Stacie N. Woolard,[6] Paul G. Thomas,[5] and Jonathan A. McCullers.[1,2,3]

[1]Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN38103; [2]Children's Foundation Research Institute, Memphis, TN 38103; [4]Laboratory of Cellular Biology, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil; Departments of [3]Infectious Diseases, [5]Immunology, and [6]Flow Cytometry, St. Jude Children's Research Hospital, Memphis, TN 38105.

Background: Eosinophils, commonly considered markers of allergic diseases, have multiple functions including pathogen defense. We found that influenza A virus infection during peak eosinophilic airways diseases reduced virus-induced weight loss and enhanced viral clearance in mice. Therefore, we hypothesized that eosinophils were promoting host immune defenses against influenza A virus.

Methods: We used transmission electron microscopy and flow cytometry to investigate eosinophil responses to viral exposure. Eosinophils were harvested from the lungs of mice subjected to a fungal asthma model and transferred to influenza virus infected mice to determine if eosinophils were able to transfer protection against influenza virus in recipient mice. $CD8^+$ T cells from virus-infected mice were co-incubated with virus peptide-pulsed eosinophils to determine whether eosinophils were able to activate $CD8^+$ T cells.

Results: Eosinophils underwent piecemeal degranulation and increased surface expression of MHCI and co-stimulatory molecules CD80 and CD86 when exposed to influenza virus. Mice that received eosinophils had reduced viral burden, improved lung compliance, and increased $CD8^+$ T cell recruitment. We also found eosinophils in the mediastinal lymph nodes in recipient mice. Virus peptide-pulsed eosinophils induced clonal expansion and interferon gamma release from $CD8^+$ T cells.

Conclusions: Eosinophils have antiviral properties against influenza A virus and may function as antigen presenting cells to enhance cellular immunity during influenza virus infections.

Grant support: Supported through the NIH NIAID CEIRS 1 N01 70005 (McCullers) and the Le Bonheur Children's Hospital Junior Faculty Award (Samarasinghe).

POSTER 72

VARIATION AT THE 17Q21 ASTHMA LOCUS IS ASSOCIATED WITH FENO LEVELS, PERIPHERAL BLOOD EOSINOPHIL COUNTS, AND EOSINOPHIL ACTIVATION IN HUMANS

Elizabeth A. Schwantes, MS*, Michael D. Evans, MS&*, Carole Ober, PhD%, Robert F. Lemanske, MD*$, Judith A. Smith, MD, PhD$ and Sameer K. Mathur, MD, PhD*#

*Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792, &Department of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792, %Department of Human Genetics, University of Chicago, Chicago, IL 60637, $Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792, #Department of Medicine, William S. Middleton Veterans Hospital, Madison, WI 53705

Background: Genome-wide association studies in children with asthma have identified single nucleotide polymorphisms (SNPs) at the 17q21 locus that confer risk for childhood onset asthma. At the rs7216389 C/T SNP, the TT genotype confers greatest risk for asthma. In mice, overexpression of one of the genes at this locus, *Ormdl3*, induced features consistent with asthma, including spon-

taneous airway remodeling, increased fibrosis, mucus production, inflammation, and methacholine induced hyper-responsiveness. In wild type mice, eosinophils (EOS) express high levels of Ormdl3, which was also shown to have a role in EOS activation.

Objectives: Examine associations between a 17q21-associated SNP, rs7216389, and eosinophilic asthma related readouts in humans, including fractional exhaled nitric oxide (FeNO), peripheral blood EOS counts, and EOS activation.

Methods: Subjects were genotyped for rs7216389. FeNO levels were measured using a NIOX MINO. Blood EOS counts were determined and EOS purified by density centrifugation, RBC hypotonic lysis, and subsequent magnetic bead negative selection (AutoMACS). EOS were stimulated with 10 ng/ml IL-5 for 4 hours and degranulation was measured by release of EOS-derived neurotoxin (EDN) (ELISA).

Results: Peripheral EOS counts were obtained from 252 genotyped subjects (46CC, 122CT, 84TT) and FeNO was measured in 157 genotyped subjects (31CC, 72CT, 54TT). The TT genotype was associated with greater FeNO (geometric mean 30.0 ppb TT, 22.3 ppb CT, 22.0 ppb CC, p=0.03) and higher rates of EOS counts greater than 200/mm^3 (45% TT, 26% CT, 24% CC, p=0.007). EOS degranulation, assessed in a subset of genotyped subjects (5TT, 5CC), revealed a trend toward increased IL-5 stimulated EDN release in TT compared to CC subjects (16.7% TT, 8.3% CC, p=0.07).

Conclusions: The asthma associated rs7216389-TT genotype at the 17q21 locus is associated with elevated FeNO, peripheral blood EOS counts, and greater IL-5 stimulated EDN release from EOS.

Grant support: P01HL088594 and P01HL070831

POSTER 73

REGULATION OF ASTHMA-SUSCEPTIBILITY ORMDL3 GENE EXPRESSION IN EOSINOPHILS AND LUNG EPITHELIAL CELLS

Sung Gil Ha, Mythili Dileepan, Xiao Na Ge, Savita P. Rao, Mathur A. Kannan and P. Sriramarao

Dept. of Veterinary & Biomedical Sciences, University of Minnesota, St. Paul, Minnesota

Background: Multiple genome-wide studies have identified Orosomucoid (yeast)-Like protein isoform 3 (ORMDL3) as a candidate asthma-susceptibility gene strongly associated with childhood and adult asthma. ORMDL3 is an endoplasmic reticulum (ER) transmembrane protein that regulates ER-mediated Ca^{2+} signaling. We previously demonstrated that ORMDL3 is expressed in eosinophils recruited to the airways of allergen-challenged mice and identified an important role for ORMDL3 in eosinophil recruitment and function in the context of allergic asthma via regulation of integrin (α4 and β2) expression and CD48-mediated degranulation. To evaluate whether ORMDL3 might serve as a target for therapeutic intervention for asthma, regulation of ORMDL3 in Eos and lung epithelial cells by microRNA (miR) was examined.

Methods: Eos were isolated from peripheral blood of normal human subjects or donors with a clinical diagnosis of allergic asthma. Murine eosinophils were obtained from bone marrow cultures of naïve wild-type mice. *ORMDL3* 3'UTR was analyzed for miRNA binding sites using target prediction algorithms/databases (Targetscan, mirnabodymap). Expression levels of ORMDL3 and miR were evaluated by q-PCR. For transient knock-down of ORMDL3 gene expression in mouse eosinophils and epithelial cells, cells were transfected with an oligonucleotide mimic of miR or a control oligonucleotide for 48 h in cell culture medium and examined by RT-PCR or confocal microscopy.

Results: Our studies show that *ORMDL3* expression in eosinophils from subjects with a clinical diagnosis of allergic asthma is higher (2-40 fold increase in expression) relative to expression in eosinophils from normal healthy donors by q-PCR. Likewise, human primary bronchial epithelial cells from an asthmatic donor express a higher level of *ORMDL3* relative to a non-asthmatic donor. Similar to mouse eosinophils, exposure of normal (non-asthmatic) human peripheral blood eosinophils to IL-3, a cytokine that promotes Eos maturation, activation and survival, induces *ORMDL3* in a dose-dependent manner. Based on miRNA target prediction data bases, miR-140-3p, which plays a role in regulation of *CD38*, a regulator of cellular Ca^{2+} dynamics in airway smooth muscle cells, has two predicted 7-mer binding sites in the 3'-UTR of the human *ORMDL3* transcript. q-PCR studies demonstrated that murine eosinophils as well as A549 and MLE12 lung epithelial cells express miR-140-3p. Transfection of eosinophils and MLE-12 cells with a mimic of miR-140-3p but not control miRNA resulted in decreased ORMDL3 expression without drastically affecting cell viability. Exposure of eosinophils to IL-3, a cytokine which induces ORMDL3 expression in these cells, inhibited miR-140-3p expression.

Conclusions: These studies suggest that miR-140-3p and potentially other miRNAs that interact with the 3'UTR of the ORMDL3 gene may play a role in regulating ORMDL3 gene expression in eosinophils and lung epithelial cells during the pathogenesis of allergic asthma.

This work was supported by NIH Grant no. HL108000.

POSTER 74

MINIMALLY INVASIVE MEASUREMENT OF INFLAMMATION IN EOSINOPHILIC ESOPHAGITIS USING AN EOSINOPHIL PEROXIDASE (EPO) BRUSH ASSAY

Hedieh Saffari,[1] Kathryn A. Peterson,[2] Leonard F. Pease,[2,3] Frederic Clayton,[4] Gerald J. Gleich,[1,5] Kristin M. Leiferman[1]

Departments of [1]Dermatology, [2]Internal medicine, Division of Gastroenterology [3]Chemical Engineering, [4]Pathology, and [5]Internal Medicine, University of Utah, Salt Lake City, UT.

Background: Eosinophils infiltrate the esophagus in eosinophilic esophagitis (EoE) and release toxic granule proteins. EoE is patchy and may remain undiagnosed, especially in patients with low eosinophil counts. Current management of EoE includes multiple expensive and time-consuming endoscopies. Studies are underway to find less invasive diagnostic methods, ways to decrease costs and means to increase treatment compliance. Previous studies showed that most eosinophils in EoE undergo cytolysis, indicating that eosinophil granule protein detection may be a better indicator of disease activity than enumeration of eosinophils. The purpose of this study is to detect and quantify eosinophil peroxidase (EPO) from esophageal mucosal brush specimens using a colorimetric assay.

Methods: Esophageal mucosal brushings were collected from normal (n=5), active EoE (n=7), and resolved EoE (n=11) subjects before endoscopic biopsy collection. Brushes were stored at -80ºC and then developed colorimetrically for 30 minutes. Absorbance (at 492 nm) was compared to average and peak eosinophil counts obtained from histopathological examination of esophageal biopsies. Results: Colorimetric development ranged from light yellow to dark brown as EPO concentration increased. EoE brush specimens yielded a distinct dark brown color of >1.2 absorbance units, whereas the majority of normal and resolved EoE brush specimens yielded a light to dark yellow color (see Table). The sensitivity and specificity of the EPO assay for differentiating EoE from resolved or normal esophagi were 100% and 93.75%, respectively ($p<0.0001$). The results strongly correlated with average eosinophil counts throughout the esophagus.

Conclusion: This proof-of-principle test demonstrates that eosinophil density correlates with EPO absorbance measurements and that eosinophil-infiltrated tissue can be distinguished from eosinophil-poor tissue by a colorimetric assay based on an esophageal mucosal brush specimen. Because EoE commonly is patchy, eosinophils counts from biopsy specimens may not reflect disease activity. Notably, our findings identified one patient with histopathologically resolved EoE (<15 eosinophils per high power field), but a positive reaction by EPO assay (absorbance of 1.737), indicating possible persistent active disease despite low eosinophil counts. These results introduce a new minimally invasive and rapid method to detect and monitor EoE disease activity. Further validation is underway with bedside experiments.

Grant support: American Partnership For Eosinophilic Disorders (APFED)

POSTER 75

IS PERIOSTIN A GOOD BIOMARKER FOR ASTHMA?

Marie-Chantal Larose, Véronique Provost, Jamila Chakir, Michel Laviolette and Nicolas Flamand. Centre de recherche de l'IUCPQ, Faculté de médecine, Université Laval, Québec, QC G1V 4G5, Canada.

Background: Periostin is increasingly recognized as a biomarker notably in atopic dermatitis. A recent study underscored that serum periostin was one of the best biomarker of severe asthmatics with persistent airway eosinophils and symptomatically uncontrolled despite high doses of corticosteroids. Given the latter study only focused on severe asthmatics, we investigated if there was a correlation between periostin and asthma severity, using the American Thoracic Society criteria.

Methods: Plasma samples were obtained from healthy subjects, atopic dermatitis subjects, moderate asthmatics with atopic dermatitis and asthmatics (mild, moderate, severe non- eosinophilic controlled, severe eosinophilic uncontrolled asthmatics). Periostin was quantitated in plasma samples by ELISA. Human primary bronchial epithelial cells (BECs) isolated from bronchial biopsies were obtained from healthy subjects and subjects with mild or severe asthma presenting high level of eosinophil counts in their induced sputum (mean 28%, range 7% - 82%). Periostin and CCL26 level were quantitated by ELISA in BECs stimulated with IL-13.

Results: Plasma periostin levels were higher in healthy subjects than mild, moderate, severe controlled and severe uncontrolled asthmatics. The decrease of plasma periostin levels observed was statistically significant between healthy subjects and moderate asthmatics ($p=0.016$). Additionally, atopic dermatitis subjects have greater plasma periostin levels compared to healthy subjects, moderate asthmatics ($p<0.0001$) and moderate asthmatics with atopic dermatitis ($p=0.0061$). The level of plasma periostin in moderate asthmatics was similar in subjects with or without atopic dermatitis. No correlation was found between plasma periostin levels and blood eosinophils ($R2=0.007$, $p=0.41$) or serum IgE levels ($R2=0.094$, $p=0.071$). Even if our results indicate that plasma periostin level is not a reliable systemic biomarker for asthma severity, periostin could still be a player in asthma and its severity. Thus, we evaluated periostin production by BECs from subjects with different asthma severity. IL-13 increased the production of

periostin by BECs over a 24 hours period, similarly to what we observed for CCL26. Of note, there was a significant correlation between CCL26 and periostin release (R2=0.53, p=0.0072).

Conclusions. Our results in plasma samples suggest that plasma periostin level is not a good systemic biomarker in asthma. Corticosteroids intake by moderate and severe asthmatics decrease plasma periostin levels. Also, subjects with atopic dermatitis have significantly greater plasma periostin levels than subjects with asthma. Although, our results with BECs suggest a role of periostin in asthma. Since lower plasma periostin levels are found in severe asthmatics vs healthy, the use of periostin as a biomarker, when investigating severe eosinophilic airway diseases, will be challenging.

Grant support: supported by a grant to ML and NF from the Fondation de l'IUCPQ and the JD- Bégin Research Chair, and scholarships to MCL from La fondation Wilbrod-Bhérer et Joseph- Demers de l'Université Laval, la direction de la recherche universitaire de l'IUCPQ and the CIHR-Quebec Respiratory Health Network Training Program. NF was supported by a salary award from the Fonds de la recherche du Québec-Santé.

POSTER 76

COMPLETE REVERSAL OF CNS MANIFESTATIONS AND SUSTAINED MOLECULAR RESPONSE FOR 6 YEARS IN CHRONIC EOSINOPHILIC LEUKAEMIA (CEL) FIP1L1-PDGFRA+ AFTER IMATINIB THERAPY.

Kiran Mathur[1], Nicholas.C.P. Cross[2], Finella Brito-Babapulle[1]

Dept of Haematology, Broomfield Hospital, Essex UK[1] , Wessex Regional Genetics Laboratory, Salisbury UK[2]

Patients with CEL present with dermatologic (37%), lungs (25%) and GIT (14%) manifestations. Cardiac (5%) and neurological (4%) presentation are relatively rare. We report a case of CEL presenting with CNS manifestations in which a complete, sustained and continuing molecular and clinical response was seen for 6 years with low dose Imatinib mesylate therapy.

A 72-years old man presented with slurred speech and double vision following a prolonged period of unresponsiveness. He had loss of recent memory. Another episode of unresponsiveness occurred the same day. Neurological examination/CT brain/carotid duplex was not conclusive. ECG was in sinus rhythm. A diagnosis of transient ischaemic attacks (TIA) was made and low dose aspirin started. Mild leucocytosis of $13x10^9$/l with eosinophilia of $3x10^9$/l was present. Haemoglobin (Hb) 13g/l and platelets $130x10^9$/l. No treatment for eosinophilia was given.

3 years later following a history of "funny turns" & loss of memory, neurological examination/ carotid duplex were not conclusive. EEG showed no epileptiform features. MRI brain showed patchy signal changes in left temporal lobe with areas of low signal surrounded by high signal on the FLAIR system. A similar appearance was seen in the right periventricular deep white matter in keeping with old areas of ischaemia/infarction. ECHO did not report mural thrombi. Hb 10.9g/l, wbc 13.7 $x10^9$/l, platelets 153 $x10^9$/l and marked eosinophilia of $9.0x10^9$/l with splenomegaly (6 cm). Bone marrow showed predominant eosinophilic precursors with no increase in blasts or involvement by lymphoma. Conventional G-banding revealed normal karyotype in all metaphases, interphase FISH analysis detected low level of FIP1L1-PDGFRA rearrangement (17/150) characteristic of myeloproliferative neoplasm with eosinophilia. FISH testing was negative for 5q33, corresponding to PDGFRB gene rearrangements. Translocation involving FGFR1 on chromosome 8p11 was not identified. Absence of KIT Asp816Val mutation by allele-specific polymerase chain reaction (PCR) ruled out systemic mastocytosis.

Imatinib mesylate, a tyrosine kinase inhibitor (TKI) which inhibits FIP1L1-PDGFRA was started and no further TIA's occurred. His memory improved. RT-PCR for FIP1L-PDGFRA after 14 months of initial treatment and 22 and 28 months later was negative showing complete molecular response. The FIP1LI-PDGFRA clone was demonstrated again after 40 months of maintenance therapy and remained positive after 9 months of 100 mg daily due to poor compliance. Imatinib dose was increased to 200 mg daily. RT-PCR became negative again demonstrating complete molecular response. He continues on 200 mg of Imatinib daily and in molecular remission 6 years from starting it and having had CEL for 9 years at least. No further CNS manifestations have occurred.

Conclusion: Neurological manifestations in patients with CEL which result from thrombi, haemorrhage, encephalopathy or peripheral neuropathy may be irreversible without treatment. In our case, we presume temporal lobe infarction to be due to eosinophil granule cationic protein neurotoxicity as there was no evidence of mural thrombus in his ECHO and complete reversal occurred with TKI treatment. in our case TKI treatment could not be stopped as FIP1L1-PDGFRA recurred with blood eosinophilia on cessation of therapy.

POSTER 77

ZINC OXIDE NANOPARTICLES CAN ALTER THE BIOLOGY OF HUMAN EOSINOPHILS

Luis Rafael Silva[1] & Denis Girard[1]

[1]Laboratoire de recherche en inflammation et physiologie des granulocytes, INRS-Institut Armand-Frappier, Université du Québec, Laval, Canada

Nanoparticles (NPs) are nanoscale materials found in a large variety of common household and workplace materials. Their use in industry has grown rapidly, leading to an increased potential exposure of humans to NPs. Studies in rodent models have shown that NPs exacerbate asthma symptoms and increase eosinophil recruitment to the lungs. Furthermore, due to their size, NPs could accumulate deeply in the lungs, where they may interact with eosinophils. We and others have demonstrated that some types of NPs can have an impact on the biology of human neutrophils altering, for example, their spontaneous apoptotic rate. Our previous studies have shown that NPs have a direct effect on granulocyte neutrophil functions; different NPs either induced or delayed spontaneous apoptosis. More recently, we demonstrated that some NPs can induce endoplasmic reticulum (ER) stress in association with inflammasome activation in human mononuclear cells. This was evidenced by the activation of the three ER stress sensors: the phosphorylation of both Inositol requiring 1-α (IRE-1α) and double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) and degradation of Activating transcription factor 6 (ATF-6) confirmed the ER stress while caspase-1 processing/activation and the production of IL-1β revealed inflammasome activation. The aim of the present study was thus to determine if NPs could also alter the biology of human eosinophils, a poorly documented area of research. We investigated this by using freshly isolated human eosinophils which we incubated with increasing concentrations of different types of NPs from 1-48 hours. Different *in vitro* assays were then performed to assess how NPs might affect eosinophils: Size and granularity were measured by flow cytometry while apoptosis was assessed by cytology and by flow cytometry using annexin-V/propidium iodide staining. Culture supernatants were also collected to measure cytokine production by ELISA and gelatinase activity with zymography. Reactive oxygen species (ROS) production was evaluated with the H2CFDA probe and measured by flow cytometry. *De novo* protein synthesis was visualized by SDS-PAGE after metabolic cell labeling. Finally, ER stress events and inflammasome activation were investigated by Western blot experiments which monitored the phosphorylation of IRE-1α and PERK, the degradation of ATF-6 and caspase-1 processing. Among the panel of NPs we tested, zinc oxide (ZnO) NPs were found to delay spontaneous eosinophil apoptosis after 24h and 48h. Moreover, they induced *de novo* protein synthesis after 24h and 48h. Finally, ZnO NPs induced IL-1β and IL-8 production in addition to the phosphorylation of PERK and degradation of ATF-6 and processing of caspase-1. To conclude, we demonstrate that human eosinophil are direct targets of NPs. Thus, their potential effect on eosinophils during the development of therapeutic strategies using NPs has to be taken under consideration; in particular, in pathologies like asthma, where the involvement of these granulocytes is well documented.

POSTER 78

STABILITY OF BLOOD EOSINOPHIL COUNT AND FENO IN HUMAN SUBJECTS

Paul S. Fichtinger, BS*, Elizabeth A. Schwantes, MS*, Michelle Wolff, MSN*, Evelyn Falibene, BS*, Maranda Hyde, BA*, Holly Eversoll, BSN*, Michael D. Evans, MS* and Sameer K. Mathur, MD, PhD*#

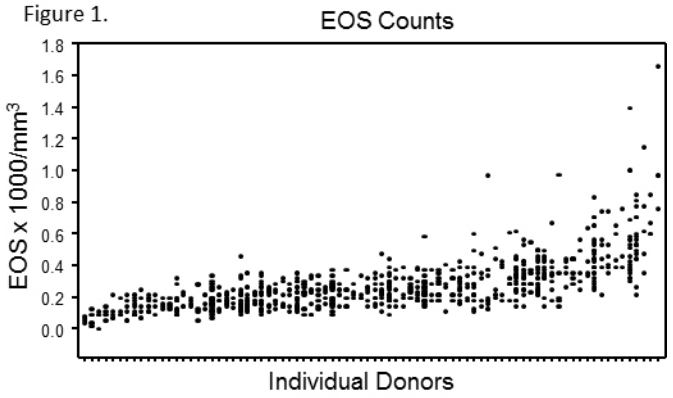
*Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI 53792 #Department of Medicine, William S. Middleton Veterans Hospital, Madison, WI 53705

Background: Recent novel asthma therapeutics, such as monoclonal antibodies or soluble receptors that block IL-5 or IL-13, have shown benefit in patients with Th2 endotype or eosinophilic asthma. Thus, there is a critical need for stable biomarkers to identify these patients. Both the blood eosinophil (EOS) count and FeNO level are frequently used as biomarkers in this capacity. Our goal was to assess the stability of using blood EOS count and FeNO level in individuals with allergic rhinitis and/or asthma.

Methods: Our EOS Laboratory Core Blood Donor Protocol currently has 451 subjects enrolled with extensive patient characterization. At each visit, FeNO is measured and a blood EOS count is determined. We selected donors with more than 4 visits (n=82) and

categorized them by allergic rhinitis (AR) and asthma (AS) status. Variability of the blood EOS counts (Figure 1) and FeNO levels was assessed.

Figure 1.



Results: Patients with AR+/AS- (n=19), AR-/AS+ (n=11), and AR+/AS+ (n=52) had mean EOS counts and coefficients of variance (CV) of 174/mm^3 and 42.1%, 254/mm^3 and 35.4%, and 244/mm^3 and 39.7%, respectively. The mean FeNO levels and CV were 22 ppb and 30.5%, 32.9 ppb and 41.7%, and 33 ppb and 38.7%, respectively. EOS counts between AR+/AS- vs AR-/AS+ (p=0.05) and AR+/AS- vs AR+/AS+ (p=0.01) were significantly different. FeNO levels between AR+/AS- and AR+/AS+ (p=0.05) were significantly different. Overall, CV for EOS counts was 39.7% (with no significant difference between groups) and for FeNO levels was 37.4% (p=0.002 between groups).

Conclusion: The use of blood EOS count or FeNO level as biomarkers must be considered with caution due to the significant variability observed in subjects over time.

Grant support: P01HL088594

POSTER 79

IDENTIFICATION OF DEGRANULATION PATTERNS AND VESICULAR TRANSPORT OF MAJOR BASIC PROTEIN IN EOSINOPHILS TRIGGERED BY THE EXPERIMENTAL SCHISTOSOMA MANSONI INFECTION

Kátia B. Amaral[1*], Felipe F. Dias[1*], Kássia K. Malta[1], Thiago P. Silva[1], Peter F. Weller[2] and Rossana C.N. Melo[1, 2]

[1] Laboratory of Cellular Biology, Department of Biology, Federal University of Juiz de Fora, MG, Brazil. [2]Division of Allergy and inflammation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School Boston, Massachusetts, USA.

Background: Eosinophils have been long associated with helminthic infections, although their functions in these diseases remain unclear. During Schistosomiasis, a chronic disease caused by the trematoda *Schistosoma mansoni*, eosinophils are specifically recruited and migrate to sites of granulomatous response where they degranulate. However, little is known on the mechanisms of eosinophil secretion during this disease. In this work, we investigate the degranulation patterns including the cellular mechanisms of major basic protein-1 (MBP-1) release from eosinophils in a mouse model of *S. mansoni* infection.

Methods: Female Swiss mice (n=6) were infected with 100 cercariae of *S. mansoni* percutaneously. Animals were euthanized after 55 days post-infection (acute phase) and liver fragments were processed for histologic analyses, conventional transmission electron microscopy (TEM), and immunogold electron microscopy (EM) using a pre-embedding approach for detection of MBP-1.

Results: A well-characterized granulomatous inflammatory response with a high number of infiltrating eosinophils surrounding the *S. mansoni* eggs was observed in the hepatic granulomas of infected mice. TEM analyses of 141 cells and a total of 3120 granules revealed distinct eosinophil degranulation processes: cytolysis (9.6%), classical and/or compound exocytosis indicated by the presence of fused granules (28.4%), and mainly piecemeal degranulation (PMD) (61.9%). PMD was identified by morphological changes of specific granules (enlargement, reduced electron-density, core disarrangement and coarse granule matrix) and presence of a high number of cytoplasmic vesicles, indicative of a vesicle-mediated transport of granule-stored products. Immunogold EM showed a consistent labeling for MBP associated with secretory granules. Moreover, extragranular sites of MBP were identified. MBP was present within vesicles distributed in the cytoplasm and attached to or surrounding the surface of emptying granules. Extracellular MBP deposition was also observed.

Conclusions: Our data demonstrate that eosinophils are able to degranulate through different patterns during the acute experimental *S. mansoni* infection, being PMD the predominant mechanism of eosinophil secretion. This means that the release of eosinophil products is occurring mainly through differential mobilization and selective secretion of granule-derived products in

response to the infection without spending whole granules, thereby maintaining intracellular granules competent for subsequent rounds of degranulation. This may be indicative of an immunomodulatory contribution to the disease process. Moreover, our study demonstrates, for the first time, a vesicular trafficking of MBP-1 within eosinophils elicited by the helminth infection. These extracellular sites may be relevant for the rapid release of small concentrations of MBP under cell activation. However, the impact of the selective secretion of MBP-1 on the direct defense against the parasite or regulation of other immune responses awaits further investigation.

Grant support: CNPq 469995/2014-9, Ministry of Health and Fapemig (Brazil) and NIH R37-AI020241, R01-051645 (USA). *These authors contribute equally to this work.

POSTER 80

INCREASED DETECTION OF ESOPHAGEAL INFLAMMATION BUT NOT REMODELING BY ENDOSCOPY COMPARED WITH RADIOLOGIC IMAGING IN ADULTS WITH EOSINOPHILIC ESOPHAGITIS

Matthew J. Nelson[1], Frank Miller[2], Nelson Moy[3], Angelika Zalewski[1], Ikuo Hirano[1]

[1] Department of Medicine, Northwestern University Feinberg School of Medicine, [2] Department of Radiology, Northwestern University Feinberg School of Medicine, [3] Department of Gastroenterology, Carle Physician Group

Background: Eosinophil predominant mucosal inflammation is central to the diagnosis and activity assessment of eosinophilic esophagitis. Esophageal mural remodeling is an important consequence of EoE that is responsible for complications of dysphagia, food impaction and esophageal stenosis in adult patients. The aim of this study was to compare the detection of esophageal inflammation and remodeling by upper endoscopy (EGD) with barium upper gastrointestinal study (UGI) in adults with EoE.

Methods: A retrospective review was performed on a single center database of adults with confirmed EoE to identify those with EGD and UGI performed within 6 months of each other. Patients included fulfilled 2011 EoE consensus recommendation criteria for EoE. Patients were excluded if they had EGD with dilation prior to UGI. UGI and EGD data were reviewed for both mucosal inflammatory and remodeling abnormalities. A prospective un-blinded, re-evaluation of the UGI images was conducted by a senior GI radiologist. EGD features were compared with both the retrospective and prospective re-evaluation of UGI studies. Statistical analysis was performed using McNemar's test.

Results: 70 patients were included with mean age 40 and slight male predominance (51%). Mean time between UGI and EGD was 41 days. Initial UGI results were classified into 5 categories: normal (23%), suggestive of EoE (rings, furrows, narrow caliber, exudates) (10%), possibly consistent with EoE (stricture, barium tablet retention; 39%), suggestive of reflux disease (6%), and miscellaneous (hiatal hernia/Schatzki ring/tertiary contractions; 23%). On re-evaluation of UGI studies, the detection rate of EoE features was 3.4 times higher. UGI re-evaluation demonstrated: normal (3%), suggestive of EoE (34%), possibly consistent with EoE (33%), suggestive of reflux disease (19%), and miscellaneous (11%). EGD demonstrated features of EoE in 94% with rings (71%), furrows (66%), exudates (30%), edema (17%) and stricture (40%). EGD detected characteristic EoE abnormalities in 94% of patients which was significantly greater than UGI (67%) ($p<0.01$). Inflammatory features (exudates, edema, furrows) were frequently appreciated by EGD (70%). Remodeling features (strictures, rings) were detected by UGI in 73% compared with 74% by EGD (NS). Strictures alone were more easily identified by UGI as they were found in 58% of patients compared with 40% for EGD ($p<0.05$).

Conclusions: (1) EGD and UGI have similar sensitivity for identifying the remodeling consequences of EoE. (2) Inflammatory features are better assessed on EGD compared with UGI. (3) Radiologists' awareness of EoE increases the diagnostic yield of UGI for detection of EoE features.

POSTER 81

DEVELOPMENT AND VALIDATION OF AN EASY TO USE ASSAY FOR THE ASSESSMENT OF EOSINOPHIL PEROXIDASE IN SPUTUM AS A SENSITIVE/REPRODUCIBLE BIOMARKER FOR PATIENT DIAGNOSIS

Sergei I. Ochkur[1], Cheryl A. Protheroe[2], Katie R. Zellner[1], Dana C. Colbert[2], Nancy A. Lee[2], Parameswaran Nair[3], and James J. Lee[1]

[1]Division of Pulmonary Medicine, Department of Biochemistry and Molecular Biology Mayo Clinic Arizona, [2]Division of Hematology and Oncology, Department of Biochemistry and Molecular Biology Mayo Clinic Arizona, Scottsdale, AZ, USA, [3]Department of Medicine, St. Joseph's Healthcare & McMaster University, Hamilton, ON, Canada

Rationale: Current management strategies used to guide anti-inflammatory treatments in subjects with asthma are often based on easy to perform assays representing surrogate metrics of pulmonary eosinophils (e.g., blood eosinophil counts or exhaled nitric oxide (FeNO)). Although more direct measures of airway eosinophil levels (e.g., assessment of sputum eosinophilia) are available, the complexities of these assessments have limited their wide-spread use in clinical settings. Thus, together with recent studies

showing problematic disconnects between disease severity and blood eosinophil counts and/or FeNO levels, the need of a clinically useful yet valid surrogate biomarker of pulmonary eosinophil activities exists.

Methods: Previously characterized paired anti-eosinophil peroxidase monoclonal antibodies were engineered into an easy-to-use ELISA kit for the detection and quantification of EPX in clinical settings by nominally trained allied health staff. The primary patient sample recovered was induced sputum but other minimally invasive samples were assessed, including spontaneous sputum as well as throat and nasal swabs.

Results: Systematic testing of available assay components and existing methodologies have yielded an EPX-based ELISA kit that may be stored in-house for extended periods of time prior to use as a same day point-of-care assay of patient samples by nominal trained allied health personnel using readily available instrumentation. More importantly, this assay was shown to be an accurate representation of the airway eosinophilia of asthma patients. In addition, evidence suggest that other minimally invasive patient samples (spontaneous sputum or throat/nasal swabs) may also have utility as alternative patient samples that may be quickly assessed in a clinical setting.

Conclusion: A novel EPX-based ELISA suitable for use in clinical lab settings represents an underappreciated surrogate marker tracking with airway eosinophilia and, in turn, disease severity.

This work is supported by Mayo Foundation for Medical Education and Research (Center for Individualized Medicine) and grants from National Institutes of Health [NAL (HL058723), JJL (HL065228), and JJL (HL124165)].

POSTER 82

MECHANISMS OF GLUCOCORTICOID RESISTANCE IN HES

Kindra Stokes[1], Pryscilla Yoon[1], Michelle Makiya[1], Nicole Holland-Thomas[2], Amy D. Klion[1], and Paneez Khoury[1]

[1] Human Eosinophil Section, Laboratory of Parasitic Disease, NIAID, National Institutes of Health, Bethesda, MD, [2] Clinical Parasitology Section, Laboratory of Parasitic Diseases, Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

Background: Glucocorticoids (GC) are anti-inflammatory agents commonly used in the treatment of symptomatic patients with hypereosinophilic syndrome (HES). GC act globally on many different immune cells and are known to promote eosinophil apoptosis and downregulation of Th2 cytokine production. Although abnormalities of GC receptor (GR) function and elevations in *β-GR isoforms* have been implicated as potential mechanisms of GC resistance in asthma, little is known about the mechanisms of GC resistance in HES.

Methods: The study population was comprised of 19 GC-naïve subjects enrolled in a prospective study of GC response in HES and 181 subjects with HES enrolled on a clinical study of eosinophilia who had previously been treated with GC and whose response to GC was known. GC responders in both cohorts were defined as subjects whose absolute eosinophil count (AEC) declined to less than 1000/mm^3 after 0.5-1mg/kg of GC for at least 1 week. Nineteen subjects in the prospective study (14 responders, 5 non-responders) had AEC and absolute neutrophil count (ANC) assessed at baseline and after 7 days of GC administration. Serum levels of cytokines (IL-4, -5, -6, -8, -10, -13, -17, and IFN-γ) were determined by suspension array in multiplex in 31 HES subjects (21 responders, 11 non-responders) prior to initiation of GC therapy and in 7 normal donors (ND). Expression of α- and β-GR isoforms was quantified by RT-PCR in eosinophils purified prior to GC treatment in a subset of the cohort. Correlations and comparison of geometric means (GM) were performed using non-parametric statistical methods.

Results: By definition, AEC decreased significantly at day 7 in all 14 GC responders in the prospective cohort (GM 2430 to 245, $p<0.001$) and in none of the 5 non-responders (GM 12800 vs GM 9927, NS). Despite these differences in eosinophil response, a comparable rise in ANC was observed in 12/14 GC responders (GM 3.39 to 6.07, $p<0.01$) and 5/5 non-responders (GM 2.51 vs. 5.27, $p<0.01$) post treatment. The geometric mean ratio of GC β- to α-GR expression in eosinophils was also comparable between responders and non-responders (0.97 vs. 0.93, p=NS).

Pre-treatment serum IL-5 levels were significantly higher in non-responders compared to responders (GM 86.2 vs. 15.3, p=0.019). Although serum IL-8 and IL-10 levels were elevated in all HES subjects at baseline compared to ND (p=0.005, Kruskall-Wallis test), levels were comparable between responders and non-responders. There were no significant differences in other cytokine levels between any of the groups.

Conclusions: The appropriate rise in peripheral neutrophils in non-responders in response to IL-8 and GC suggests that GR abnormalities are not a global phenomenon in GC resistance in HES. Similarly, the normal ratio of GR isoforms is inconsistent with GR-β overexpression as the mechanism of GC resistance in this disorder. Elevated IL-5 levels in GC non-responders, rather than elevations

in inflammatory cytokines such as IL-6, may play a role in lack of response to GC in HES. Further characterization of cytokine and chemokine profiles, as well as assessment of receptor function and signaling pathways are clearly needed.

Grant support: This study was funded by the Division of Intramural Research, NIAID, NIH and by NCI Contract No. HHSN261200800001E. This research was supported in part by the National Institute of Allergy and Infectious Diseases.

Participant List

As of 26 June 2015

A

Aceves, Seema, University of California, San Diego, San Diego, CA, United States, saceves@ucsd.edu

Ackerman, Steven, University of Illinois at Chicago, Chicago, IL, United States, sackerma@uic.edu

Adamko, Darryl, University of Saskatchewan, Saskatoon, SK, Canada, darryl.adamko@usask.ca

Akuthota, Praveen, Beth Israel Deaconess Medical Center, Boston, MA, United States, pakuthot@bidmc.harvard.edu

Alam, Rafeul, National Jewish Health, Denver, CO, United States, AlamR@NJHealth.org

Andersson, Jennie, University of Gothenburg, Gothenburg, Sweden, jennie.andersson@microbio.gu.se

Arron, Joe, Genentech, South San Francisco, CA, arron.joseph@gene.com

B

Ben-Baruch-Morgenstern, Netali, Tel Aviv University, Tel Aviv, Israel, netalimorg@gmail.com

Bettigole, Sarah, Harvard University, New York, NY, United States, sab2051@med.cornell.edu

Blom, Kristin, Uppsala University, Uppsala, Sweden, kristin.blom@medsci.uu.se

Bochner, Bruce, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, bruce.bochner@northwestern.edu

Bolus, William, Vanderbilt University Medical Center, Nashville, TN, United States, w.r.bolus@gmail.com

Bozik, Michael, Knopp Biosciences LLC, Pittsburgh, PA, United States, michael.bozik@knoppbio.com

Bozza, Patricia, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, pbozza@ioc.fiocruz.br

Brito-Babapulle, Finella, Broomfield Hospital, Richmond, Surrey, United Kingdom, theonlyfbinhaem@gmail.com

Broide, David, UC San Diego, La Jolla, CA, United States, dbroide@ucsd.edu

Bryce, Paul, Northwestern University Feinberg School of Medicine, Chicago, Ill, United States, p-bryce@northwestern.edu

Busse, William, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States, wwb@medicine.wisc.edu

C

Caldwell, Julie, Cincinnati Children's Hospital, Cincinnati, OH, United States, julie.caldwell1@cchmc.org

Catalano, Alberto, Royal Prince Alfred Hospital, Camperdown, NSW, Australia, catalano.alberto@gmail.com

Cheng, Edaire, University of Texas Southwestern Medical Center, Dallas, TX, United States, edaire.cheng@utsouthwestern.edu

Cianferoni, Antonella, The children's hospital of Philadelphia, Philadelphia, PA, United States, cianferonia@email.chop.edu

Cihakova, Daniela, Johns Hopkins University, Baltimore, MD, United States, dcihako1@jhmi.edu

Collins, Margaret, Cincinnati Children's Hospital, Cincinnati, OH, United States, margaret.collins@cchmc.org

Cook-Mills, Joan, Northwestern University, Chicago, IL, United States, j-cook-mills@northwestern.edu

Cools, Jan, Center for Human Genetics, Leuven, Belgium, jan.cools@cme.vib-kuleuven.be

D

Davoine, Francis, University of Alberta, Edmonton, AB, Canada, fdavoine@ualberta.ca

Dellon, Evan, University of North Carolina - Chapel Hill, Chapel Hill, NC, United States, edellon@med.unc.edu

Diny, Nicola, Johns Hopkins University, Baltimore, MD, United States, ndiny1@jhu.edu

Doherty, Taylor, UC San Diego, La Jolla, CA, United States, tdoherty@ucsd.edu

Dworetzky, Steven, Knopp Biosciences, Pittsburgh, PA, United States, dworetzky@knoppbio.com

Dyer, Kimberly, NIH - IIS/LAD/NIAID, Bethesda, MD, United States, kdyer@niaid.nih.gov

E

Esnault, Stephane, University of Wisconsin, Madison, WI, United States, sesnault@wisc.edu

F

Falahati, Rustom, Allakos, San Carlos, CA, United States, rustom.falahati@gmail.com

Falk, Gary, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States, gary.falk@uphs.upenn.edu

Farahi, Neda, University of Cambridge, Cambridge, United Kingdom, nf231@medschl.cam.ac.uk

Fernandes, Steve, Johns Hopkins Medical Institution, Baltimore, MD, United States, lidpeg@gmail.com

Fichtinger, Paul, University of Wisconsin, Madison, WI, United States, psfichtinger@medicine.wisc.edu

Fillon, Sophie, University of CO - Denver, Aurora, CO, United States, sophie.fillon@ucdenver.edu

Flamand, Nicolas, Université Laval, Québec City, QC, Canada, nicolas.flamand@criucpq.ulaval.ca

Fosbury, Hugh, Teva Pharmaceuticals, Frazer, PA, United States, caroline.moore02@tevapharm.com

Participant List

Frei, Robert, Medical University of Graz, Graz, Austria, robert.frei@medunigraz.at

Fryer, Allison, Oregon Health and Science University, Portland, OR, United States, allison.fryer@gmail.com

Fulkerson, Patricia, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, patricia.fulkerson@cchmc.org

Furuta, Glenn, University of Colorado School of Medicine, Aurora, CO, United States, glenn.furuta@childrenscolorado.org

G

Gangwar, Roopesh, Hebrew University of Jerusalem, Jerusalem, Israel, gangwarroop@gmail.com

Gauvreau, Gail, McMaster University, Caledonia, ON, Canada, gauvreau@mcmaster.ca

Ge, Xiaona, University of Minnesota, St. Paul, MN, United States, gexn@umn.edu

Gela, Anele, Lund University, Lund, Sweden, anele.gela@med.lu.se

Gleich, Gerald, University of Utah, Salt Lake City, UT, United States, gerald.gleich@hsc.utah.edu

Gonsalves, Nirmala, Northwestern University, Chicago, IL, United States, n-gonsalves@northwestern.edu

Gupta, Sandeep, Indiana University, Zionsville, IN, United States, sgupta@iu.edu

H

Ha, Sung Gil, University of Minnesota, St. Paul, MN, United States, haxxx048@umn.edu

Haney, Elizabeth, Genentech, Nashville, TN, United States, haney.elizabeth@gene.com

Hess, Melody, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, melody.hess@cchmc.org

Hirano, Ikuo, Northwestern University, Chicago, IL, United States, i-hirano@northwestern.edu

Hogan, Simon, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, simon.hogan@cchmc.org

Hon, Emily, Riley Hospital for Children at Indiana University Health, Indianapolis, IN, United States, echon@iu.edu

Humbles, Alison, MedImmune, LLC, Gaithersburg, MD, United States, humblesa@medimmune.com

I

Islam, Sabina, Massachusetts General Hospital, Charlestown, MA, United States, sislam@mgh.harvard.edu

Itan, Michal, Tel Aviv University, Tel Aviv, Israel, itanm@post.tau.ac.il

J

Jacobsen, Elizabeth, Mayo Clinic Arizona, Scottsdale, AZ, United States, jacobsen.elizabeth@mayo.edu

Jacoby, David, Oregon Health & Science University, Portland, OR, United States, jacobyd@ohsu.edu

Janevska, Daniela, Northwestern University, Chicago, IL, United States, danielajanevska2012@u.northwestern.edu

Johansson, Kristina, University of Gothenburg, Gothenburg, Sweden, kristina.johansson.4@gu.se

Johansson, Mats, University of Wisconsin, Madison, WI, United States, mwjohansson@wisc.edu

Jönsson, Ulla-Britt, Uppsala University, Uppsala, Sweden, ulla-britt.jonsson@medsci.uu.se

Jung, YunJae, Department of Microbiology, College of Medicine, Gachon University, Incheon, Korea, yjjung@gachon.ac.kr

K

Kahn, Jean Emmanuel, Hopital Foch, Suresnes, France, je.kahn@hopital-foch.org

Kano, Gen, Kyoto Prefectural University of Medicine, Kyoto, Japan, gkano@koto.kpu-m.ac.jp

Katz, Lynn, San Mateo, CA, United States, lynnkatzcra@hotmail.com

Kelly, Elizabeth (Becky), University of Wisconsin, Madison, WI, United States, eak@medicine.wisc.edu

Khalil, Ahmed, Chicago, IL, United States, ahmedsukar_89@hotmail.com

Khoury, Paneez, National Institutes of Health, Takoma Park, MD, United States, khouryp@niaid.nih.gov

Kita, Hirohito, Mayo Clinic Rochester, Rochester, MN, United States, kita.hirohito@mayo.edu

Klion, Amy, NIAID/NIH, Baltimore, MD, United States, amy@zunkeler.com

Kodroff, Ellyn, C.U.R.E.D. (Campaign Urging Research for Eosinophilic Disease), NFP, Buffalo Grove, IL, United States,

Kolbeck, Roland, MedImmune LLC, Gaithersburg, MD, United States, kolbeckr@medimmune.com

Kuang, Fei Li, NIAID / NIH, Bethesda, MD, United States, fkuang@gmail.com

Kuhl, Jonathan, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, jonathan.kuhl@cchmc.org

Kumar, Santhosh, Virginia Commonwealth University, Richmond, VA, United States, skumar@vcu.edu

Kuritzky, Jacqueline, Regeneron Pharmaceuticals, Tarrytown, NY, United States, jacqueline.kuritzky@regeneron.com

Participant List

L

Lacy, Paige, University of Alberta, Edmonton, AB, Canada, paige.lacy@ualberta.ca

Laffey, Kimberly, University of Illinois at Chicago, Chicago, IL, United States, fgao4@uic.edu

Larose, Marie-Chantal, Université Laval, Québec, Canada, Marie-Chantal.Larose@criucpq.ulaval.ca

Lawrence, Rachel, University of London, London, United Kingdom, rlawrence@rvc.ac.uk

Lebold, Katie, Oregon Health and Science University, Portland, OR, United States, lebold@ohsu.edu

Lee, James, Mayo Clinic Arizona, Scottsdale, AZ, United States, jjlee@mayo.edu

Lefèvre, Guillaume, Regional University Hospital Lille, Levallois Perret Cedex, France, guillaume.lefevre@chru-lille.fr

Legrand, Fanny, NIAID NIH, Bethesda, MD, United States, fanny.legrand@nih.gov

Leiferman, Kristin, University of Utah Health Care, Salt Lake City, UT, United States, kristin.leiferman@hsc.utah.edu

Lerner, Diana, Medical College of Wisconsin, Milwaukee, WI, United States, dglerner@mcw.edu

Levi-Schaffer, Francesca, The Hebrew University of Jerusalem, Jerusalem, Israel, francescal@ekmd.huji.ac.il

Lloyd, Clare, Imperial College, London, United Kingdom, c.lloyd@imperial.ac.uk

Loutsios, Chrystalla, University of Cambridge, Cambridge, United Kingdom, chrystalla_1@hotmail.com

Lutter, Rene, AMC/University of Amsterdam, Amsterdam, Netherlands, r.lutter@amc.uva.nl

M

Mack, Ethan, University of Pennsylvania, Philadelphia, PA, United States, etmack@mail.med.upenn.edu

Makiya, Michelle, National Institutes of Health, Bethesda, MD, United States, makiyam@niaid.nih.gov

Masterson, Joanne, University of Colorado Denver, Aurora, CO, United States, Joanne.Masterson@ucdenver.edu

Mathur, Sameer, University of Wisconsin, Madison, WI, United States, sm4@medicine.wisc.edu

Matsumoto, Kenji, National Research Institute for Child Health and Development, Tokyo, Japan, matsumoto-k@ncchd.go.jp

Melo, Rossana, Federal University of Juiz de Fora, Juiz de Fora, Brazil, rossana.melo@ufjf.edu.br

Milner, Joshua, NIH NIAID, Bethesda, MD, United States, jdmilner@niaid.nih.gov

Mingler, Melissa, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, melissa.mingler@cchmc.org

Morello, Jean-Pierre, Teva Canada Innovation, Montreal, QC, Canada, jean-pierre.morello@tevapharm.com

Moreno, Liliana, Northwestern University, Chicago, IL, United States, liliana.moreno@northwestern.edu

Morris, David, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, david.morris@cchmc.org

Mosher, Deane, University of Wisconsin, Madison, WI, United States, dfmosher@wisc.edu

Moshkovits, Itay, Tel-Aviv University, Lod, Israel, itaymoshko@gmail.com

Muir, Amanda, The Children's Hospital of Philadelphia, Philadelphia, PA, United States, muira@email.chop.edu

Mukherjee, Manali, McMaster University, Hamilton, ON, Canada, mukherj@mcmaster.ca

Mukkada, Vincent, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, vmukkada@gmail.com

Munitz, Ariel, Tel Aviv University, Tel Aviv, Israel, arielm@post.tau.ac.il

N

Nair, Parameswaran, McMaster University, Hamilton, ON, Canada, parames@mcmaster.ca

Nazaroff, Christopher, Mayo Clinic in Arizona, Scottsdale, AZ, United States, nazaroff.christopher@mayo.edu

Neves, Josiane, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, josiane.sabbadini.neves@icb.ufrj.br

Newbold, Paul, MedImmune, Gaithersburg, MD, United States, newboldp@medimmune.com

Nguyen, Nathalie, Children's Hospital Colorado, Aurora, CO, United States, nathalie.nguyen@childrenscolorado.org

Nussbaum, Jesse, UCSF School of Medicine, San Francisco, CA, United States, jesse.nussbaum@ucsf.edu

O

O'Sullivan, Jeremy, Northwestern University, Chicago, IL, United States, jeremy.osullivan@northwestern.edu

Ochkur, Sergei, Mayo Clinic Arizona, Scottsdale, AZ, United States, ochkur.sergei@mayo.edu

Ogbogu, Princess, Ohio State University, Columbus, OH, United States, princess.ogbogu@osumc.edu

Olson, Ashleigh, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States, aolson@uwhealth.org

Ortega, Hector, GlaxoSmithKline, Research Triangle Park, NC, United States, hector.g.ortega@gsk.com

Participant List

P

Pazdrak, Konrad, University of Texas Medical Branch, Galveston, TX, United States, kopazdra@utmb.edu

Peinhaupt, Miriam, Medical University of Graz, Graz, Austria, miriam.peinhaupt@medunigraz.at

Pesek, Robbie, Arkansas Children's Hospital, Little Rock, AR, United States, rdpesek@uams.edu

Peterson, Kathryn, University of Utah, Salt Lake City, UT, United States, kathryn.peterson@hsc.utah.edu

Phadke, Neena, Chicago, IL, United States, neena.phadke@astrazeneca.com

Picard, Alain, Teva Canada Innovation, Montreal, QC, Canada, alain.picard@tevapharm.com

Pothoven, Kathryn, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, k-pothoven@u.northwestern.edu

Prakash Babu, Senbagavalli, National Institutes of Health, Bethesda, MD, United States, prakashs2@niaid.nih.gov

Prizment, Anna, University of Minnesota, Minneapolis, MN, United States, prizm001@umn.edu

Prussin, Calman, NIAID/NIH, Rockville, MD, United States, calmanp@gmail.com

R

Radin, Allen, Regeneron Pharmaceuticals Inc., Tarrytown, NY, United States, allen.radin@regeneron.com

Radinger, Madeleine, University of Gothenburg, Göteborg, Sweden, madeleine.radinger@gu.se

Regan, Jennifer, Northwestern, Chicago, IL, United States, jregan004@gmail.com

Reichman, Hadar, Tel-Aviv University, Tel Aviv, Israel, hadarsen@gmail.com

Rice, Tyler, NIAID NIH, Bethesda, MD, United States, tyler.rice@nih.gov

Roth, Diane, Regeneron, Tarrytown, NY, United States, diane.roth@regeneron.com

Roth-Carter, Quinn, Oregon Health & Science Univeristy, Porltand, OR, United States, rothcart@ohsu.edu

Rothenberg, Marc, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, marc.rothenberg@cchmc.org

Roufosse, Florence, Hôpital Erasme (ULB), Brussels, Belgium, froufoss@ulb.ac.be

Rubin, Jenny, Uppsala University, Uppsala, Sweden, jenny.rubin@medsci.uu.se

S

Sable, Kathleen, APFED, krsable@bellsouth.net

Sabogal Pineros, Yanaika, Amsterdam Medical Center, Amsterdam, Netherlands, yashabo@hotmail.com

Safroneeva, Ekaterina, Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland, ekaterina.safroneeva@ispm.unibe.ch

Samarasinghe, Amali, University of Tennessee Health Science Center, Memphis, TN, United States, asamaras@uthsc.edu

Schleimer, Robert, Northwestern Feinberg School of Medicine, Chicago, IL, United States, rpschleimer@northwestern.edu

Schnaar, Ronald, Johns Hopkins University School of Medicine, Baltimore, MD, United States, schnaar@jhu.edu

Schorer-Cortinas, Fabiola, Institute of Pharmacology, Bern, Switzerland, fabiola.schorer@pki.unibe.ch

Schroeder, Shauna, Phoenix Childrens Hospital/University of Arizona, Scottsdale, AZ, United States, shauna.schroeder@gmail.com

Schwantes, Elizabeth, University of Wisconsin-Madison, Madison, WI, United States, ed2@medicine.wisc.edu

Sehmi, Roma, McMaster University, Hamilton, ON, Canada, sehmir@mcmaster.ca

Shen, Zhong-Jian, University of Texas Southwestern Medical Center, Dallas, TX, United States, Zhong.Shen@utsouthwestern.edu

Silva, Luis, IAF-INRS, Laval, QC, Canada, luisrafael.silva@iaf.inrs.ca

Simon, Dagmar, University Hospital Bern, Bern, Switzerland, dagmar.simon@insel.ch

Simon, Hans-Uwe, University of Bern, Bern, Switzerland, hus@pki.unibe.ch

Smith, Steven, McMaster University, Waterdown, ON, Canada, smiths49@mcmaster.ca

Souza, Anselmo, MedImmune, Gaithersburg, MD, United States, anselmossouza@gmail.com

Spencer, Lisa, Beth Israel Deaconess Medical Center, Boston, MA, United States, lspencer@bidmc.harvard.edu

Sriramarao, P., University of Minnesota, Saint Paul, MN, United States, psrao@umn.edu

Stefani-Hunyady, Diana, Shire, Lexington, MA, United States, dstefani_md@yahoo.com

Stokes, Kindra, NIH/NIAID, Bethesda, MD, United States, kindra.stokes@nih.gov

Strandmark, Julia, Freie University, Berlin, Germany, julia.strandmark@fu-berlin.de

Sturm, Eva, Medical University of Graz, Graz, Austria, eva.sturm@medunigraz.at

Participant List

T

Tokunaga, Takahiro, University of Fukui, Japan, Yoshida-gun, Japan, t-toku3@nifty.com

Tomasevic, Nenad, Allakos, Inc., San Carlos, CA, United States, ntomasevic@allakos.com

Toor, Iqbal, University of Edinburgh, Edinburgh, United Kingdom, itoor@staffmail.ed.ac.uk

Travers, Jared, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, Jared.Travers@cchmc.org

Turton, Keren, University of Wisconsin-Madison, Madison, WI, United States, turton@wisc.edu

U

Umetsu, Dale, Genentech, Portola Valley, CA, United States, umetsud@gene.com

W

Wang, Haibin, Beth Israel Deconess Medical Center, Boston, MA, United States, hwang1@bidmc.harvard.edu

Wardlaw, Andrew, Institute for Lung Health, University of Leicester, Leicester, , United Kingdom, aw24@leicester.ac.uk

Wechsler, Michael, National Jewish Health, Denver, CO, United States, mikewechsler@gmail.com

Weller, Peter, Beth Israel Deaconess Medical Center, Boston, MA, United States, pweller@bidmc.harvard.edu

Wen, Ting, CCHMC, Cincinnati, OH, United States, Ting.wen@cchmc.org

Wenneras, Christine, Sahlgrenska University Hospital, Goteborg, Sweden, christine.wenneras@microbio.gu.se

Wicher, Sarah, Oregon Health & Science University, Portland, OR, United States, wicher@ohsu.edu

Y

Yamada, Yoshiyuki, Gunma Children's Medical Center, Shibukawa, Gunma, Japan, yamaday@gcmc.pref.gunma.jp

Yang, Ming, Faculty of Health, University of Newcastle, Newcastle, Australia, ming.yang@newcastle.edu.au

Z

Zangrilli, James, Teva Pharmaceuticals, Frazer, PA, United States, james.zangrilli@tevapharm.com

Zheng, Tao, Yale School of Medicine, New Haven, CT, United States, tao.zheng@yale.edu

Zimmermann, Nives, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States, nives.zimmermann@cchmc.org

Zinger, Celia, Immune Pharmaceuticals, New York, NY, United States, celia.zinger@immune.com

Notes

Notes


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

Acknowledgements

IES thanks the following partners for their support of the 9th Biennial Symposium

Gold Supporters

MedImmune / AstraZeneca

TEVA Respiratory

Silver Supporters

IMMUNE Pharmaceuticals

receptos

Roche

Takeda

Bronze Supporters

Allakos

Apfed American Partnership for Eosinophilic Disorders

CURED Campaign Urging Research for Eosinophilic Disease www.CuredFoundation.org

Diagnostics Development A P&M Venge AB company

KNOPP BIOSCIENCES

TIGER

Additional Support

Novartis Institutes for BioMedical Research, Inc.

Society Sponsors

American Academy of Allergy Asthma & Immunology

EAACI European Academy of Allergy and Clinical Immunology

Speaker Travel Support

KU LEUVEN

VIB

Grant Support

National Institutes of Health

Funding for this conference was made possible [in part] by 1R13AI118098-01 from the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.