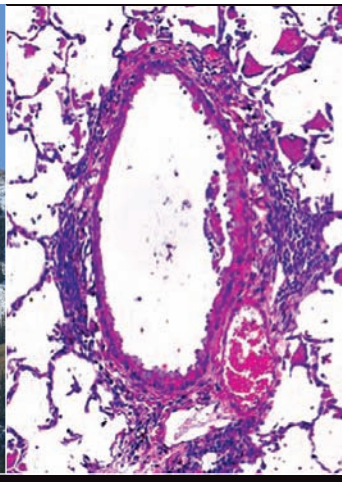
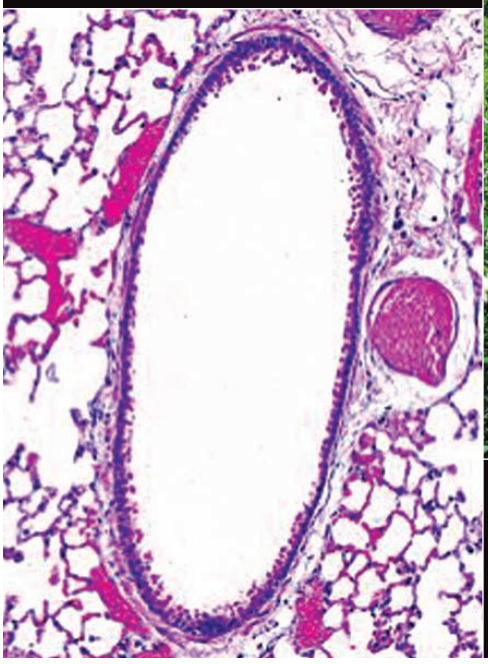


EOSINOPHIL 2007

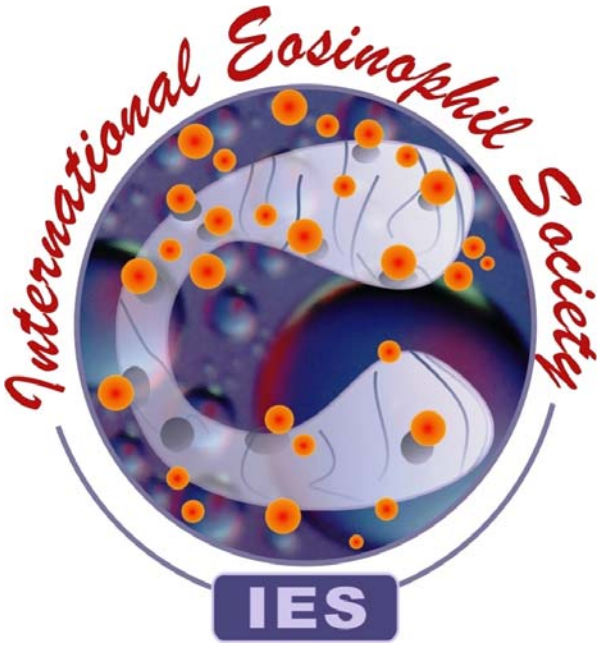
5th BIENNIAL SYMPOSIUM

International Eosinophil Society

July 18-22, 2007
Snowbird, Utah



PROGRAM



5th Biennial Symposium

International Eosinophil Society

Eosinophil 2007

July 18 – 22, 2007

Cliff Lodge, Snowbird, Utah, USA

Gerald J. Gleich – Organizer

Steven J. Ackerman – Co-organizer

James J. Lee – Scientific Program

Sanjiv Sur – Conference Website

5th BIENNIAL SYMPOSIUM, INTERNATIONAL EOSINOPHIL SOCIETY
EOSINOPHIL 2007
JULY 18-22, 2007, CLIFF LODGE, SNOWBIRD, UTAH USA

Dear Participants,

We welcome you to the beautiful Little Cottonwood Canyon and the Cliff Lodge at Snowbird.

This is a timely gathering of scientists and clinician-investigators who pursue research on the eosinophil, both normal functions in health and pathophysiologic roles in disease. Publications in this field have risen dramatically over the last two decades. However, the precise roles of this intriguing granulocyte in the diseases with which it is associated continue to remain somewhat elusive. Over the next few days you will hear much information that will most certainly shed new light on this important question. We are also fortunate to have other affiliated or complimentary meetings occurring at Snowbird this week. These include a pre-symposium workshop on developing international collaborative clinical research trials for hypereosinophilic diseases, hosted by Amy Klion; the annual meeting of the American Partnership for Eosinophilic Diseases (APFED), a patient education and research-oriented group that will meet along side us Friday through Sunday; and the Intermountain West Allergy Association (IWAA) with whom we will hold a joint scientific session on Friday afternoon.

Our goal is to encourage open discussion on the roles of eosinophils in health and disease, during the scientific presentations, at the poster discussion sessions, and at the conclusion of the Symposium on Sunday morning. We are thrilled at the level of interest in eosinophil basic and clinical biology, as reflected by the attendance at this symposium of world-class scientists who have come together to review recent advances in molecular and cellular biology, immunobiology, and clinical dimensions of this unique leukocyte. We are likewise thrilled by the many junior and other basic and clinical investigators who will present talks and posters.

We wish to gratefully acknowledge the hard work of all of our organizing and fund-raising committees and individuals, and in particular, our corporate, institution, foundation and society sponsors for their generous financial support of the Symposium. Without them, this outstanding biennial conference would not be possible.

We also wish to express our deepest gratitude and thanks to all those individuals who helped in the organization of this enterprise, including Beth Mays and Wendy Book from the American Partnership for Eosinophilic Diseases, Jane Whitener, our conference planner at the University of Illinois, Linda Mardel, administrative assistant Dr. Jamie Lee at Mayo Clinic Scottsdale, and to all others who contributed both their time and energy for this conference.

We have purposely built into the scientific program some unscheduled time on most afternoons to provide you with an opportunity for informal interactions with your fellow participants and the 3R's – Rest, Relaxation and Recreation. We hope you will enjoy your time at Snowbird, Little Cottonwood Canyon, and the surrounding areas.

On behalf of the organizers and sponsors, welcome again to *Eosinophil 2007!*

Jerry Gleich, Steve Ackerman, Jamie Lee

Table of Contents

ACKNOWLEDGEMENTS

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

— CORPORATE SPONSORS —

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Conference Grant to Dr. Amy Klion

— FOUNDATION AND SOCIETY SPONSORS —

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The International Eosinophil Society

Intermountain West Allergy Association

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Sanjiv Sur

ACKNOWLEDGEMENTS

We thank the following individuals for their outstanding fundraising efforts on behalf of the IES and Eosinophil 2007:

Steven Ackerman

Gerald Gleich

James Lee

Amy Klion

Glenn Furuta

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University of Illinois at Chicago

Abdul Khan
University of Illinois at Chicago

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

Thanks to the following individuals for preparation of the program and abstracts book:

Steven Ackerman
University of Illinois at Chicago

Jane Whitener
Office of Conferences and Institutes, University of Illinois

SYMPOSIUM OVERVIEW

Unless otherwise indicated, all activities will take place at the Cliff Lodge.

General Sessions: Ballroom I ● Posters: Magpie Room ● Breakfast and Breaks: Ballroom I Foyer

WEDNESDAY, JULY 18

15:00 Registration Desk Opens

19:00 – 22:00 Welcome Reception and Meeting Orientation (20:00) *(Golden Cliff Room)*

22:00 Session Moderators Meeting *(Ballroom I)*

THURSDAY, JULY 19

7:00 Breakfast and Poster Setup

8.00 Scientific Session

10:45 Coffee Break and Open Poster Viewing

11:15 Scientific Session

13:15 Lunch

14:00 – 15:45 Scientific Session

Social Event: Salt Lake City Outing

16:10 Buses begin loading *(Cliff Lodge)*

16:30 Buses depart

FRIDAY, JULY 20

7:00 Breakfast

8:00 Scientific Session

10:15 Coffee Break and Open Poster Viewing

10:45 Pro-Con Debates

12.15 – 15:30 **Free Time**

15:30 Scientific Session, Joint session with IWAA

17:30 Refreshment Break

17:45 – 19:15 Poster Viewing and Discussion Sessions I & II

Social Event: BBQ Dinner/Tent Event

19:30 *(Conference Center terrace tent, Cliff Lodge, level 1)*

SATURDAY, JULY 21

7:00 Breakfast

8:00 Scientific Session

10:30 Coffee Break and Open Poster Viewing

11:00– Scientific Session

12:15 – 15:00 **Free Time**

12:15 – 14:15 Executive Committee Meeting, International Eosinophil Society *(White Pine Room)*

15:00 Scientific Session

16:00 Refreshment Break

16:15 – 18:40 Minisymposium: Paul Ehrlich Lectureships

Social Event: Symposium Banquet
(Market Street Grill, Cottonwood)

19:00 Buses begin loading *(Cliff Lodge)*

19:10 Buses depart

SUNDAY, JULY 22

7:00 Breakfast

8.00 General Scientific Session

9:00 Coffee Break

9:10 General Scientific Session

10:30 Coffee Break

10:45 General Scientific Session

11:45 Open Discussion, Future Directions

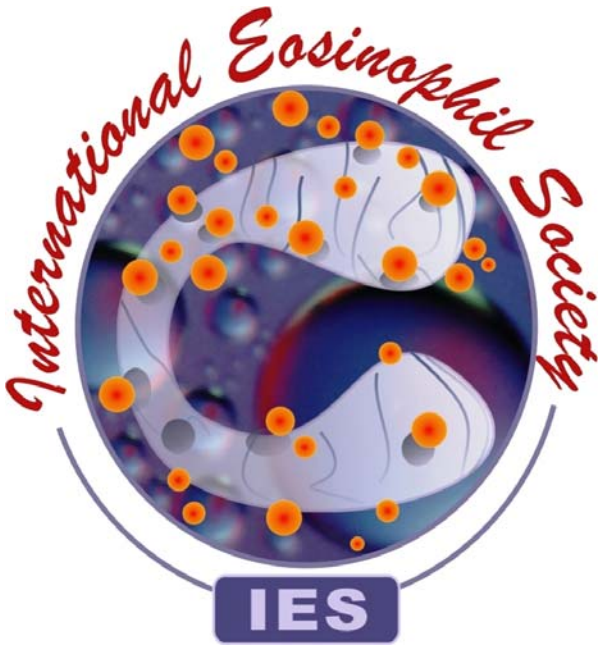
12:30 Meeting Adjourns

IN CASE OF EMERGENCY

Should you need to reach a member of the symposium organizing group during your stay at Snowbird, please note the following cell phone numbers —

Jerry Gleich: 801-949-0324

Steve Ackerman: 630-404-0333



5th Biennial Symposium

International Eosinophil Society

— Eosinophil 2007 —

Scientific Program

July 18 – 22, 2007

Cliff Lodge, Snowbird, Utah, USA

Gerald J. Gleich – Organizer

Steven J. Ackerman – Co-organizer

James J. Lee – Scientific Program

PROGRAM

WEDNESDAY, JULY 18 — AFTERNOON/EVENING

15:00 –	Check-in and Registration at Cliff Lodge, Snowbird
19:00 – 22:00	**OPENING RECEPTION** *(Golden Cliff Room)*
20:00 – 20:15	**WELCOME AND MEETING ORIENTATION** Gerald Gleich, IES President, Conference Organizers, Executive Board
22:00 – 22:30	**SESSION MODERATORS MEETING** *(Ballroom I)* All session moderators meet with James Lee

THURSDAY, JULY 19 — MORNING

Note that all general sessions throughout the Symposium will be held in Ballroom I.

7:00 – 8:00	**BREAKFAST (provided for symposium registrants)**	
SESSION: EOSINOPHIL TRAFFICKING, ACTIVATION/INHIBITION, AND SIGNAL TRANSDUCTION		
Moderators: Paul Coffer and Bruce Bochner		
8:00 – 8:30	**State of the Art Presentation – Eosinophil-mediated Signal Transduction -** Leo Koenderman (Utrecht University, Netherlands)	
8:30 – 8:45	James Malter (University of Wisconsin, Madison, USA)	Pin1 Controls Pulmonary Eosinophilic Inflammation
8:45 – 9:00	Paul Coffer (University Medical Center, Utrecht, Netherlands)	Two different faces of GSK-3: Regulation of eosinophil maturation and survival
9:00 – 9:15	**Cutting Edge: Oral Abstract Presentation** Tetsuya Adachi (Teikyo University School of Medicine, Tokyo, Japan)	Transduction of PTEN into eosinophils attenuates survival, chemotaxis and eosinophilic inflammation
9:15 – 9:45	**State of the Art Presentation – Eosinophil Trafficking, Activation, and Inhibition** Peter F. Weller (Harvard Medical School, Boston, USA)	
9:45 – 10:00	P. Srirama Rao (La Jolla Institute for Molecular Medicine, USA)	Galectin-3 and eosinophil trafficking
10:00 – 10:15	Clive Page (King's College, London, UK)	Platelets and eosinophils: A close contact relationship
10:15 – 10:30	Joan Cook-Mills (Northwestern University, Chicago, USA)	Non-hematopoietic NADPH oxidase regulation of lung eosinophilia and airway hyperresponsiveness in experimentally-induced asthma
10:30 – 10:45	Bruce Bochner (Johns Hopkins University, Baltimore, USA)	Localization and characterization of glycan ligands for Siglec-F, the murine paralog of the eosinophil inhibitory receptor Siglec-8
10:45 – 11:15	**COFFEE BREAK**	

PROGRAM

THURSDAY, JULY 19 — MORNING *(continued)*

SESSION: EOSINOPHIL EFFECTOR FUNCTIONS		
Moderators: Allison Fryer and Hans-Uwe Simon		
11:15 – 11:45	**State of the Art Presentation** Gerald Gleich (University of Utah, Salt Lake City, USA)	
11:45 – 12:00	Hans-Uwe Simon (University of Bern School of Medicine, Switzerland)	Extracellular traps generated by granulocytes– Effector function of viable cells or result of a new form of cell death?
12:00 – 12:15	Allison Fryer (Oregon Health Sciences University, Portland, USA)	Eosinophils and parasympathetic nerve function in the lung
12:15 – 12:30	Sameer K. Mathur (University of Wisconsin, Madison, USA)	Eosinophil regulation of Th2 cytokine secretion from CD4+ T-cells is dependent on ICAM-1
12:30 – 12:45	Paige Lacy (University of Alberta, Edmonton, Canada)	Eosinophil superoxide release and airway hyperresponsiveness are not dependent on Rac2 GTPase
12:45 – 13:00	Gerd Döring (University of Tübingen, Germany)	Post-translational tyrosine nitration of eosinophil granule toxins mediates improved killing of Trypanosoma cruzi
13:00 – 13:15	**Cutting Edge: Oral Abstract Presentation** Josiane S. Neves (Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, USA)	Eosinophil granules function extracellularly as receptor-mediated secretory organelles
13:15 – 14:00	**LUNCH (provided for symposium registrants)**	

PROGRAM

THURSDAY, JULY 19 — AFTERNOON/EVENING

SESSION: EOSINOPHILS AS REGULATORS OF PHYSIOLOGICAL HOMEOSTASIS — HOST-PARASITE INTERACTIONS		
Moderators: Helene Rosenberg and Per Venge		
14:00 – 14:30	**State of the Art Presentation** Monique Capron (Institut Pasteur de Lille, France)	
14:30 – 14:45	Tom Nutman (NIAID, NIH, Bethesda, USA)	Parasite-encoded IL-5 receptor binding protein illustrates a strategy used by helminth parasites for immune evasion
14:45 – 15:00	Per Venge (University of Uppsala, Sweden)	ECP and EPX in parasitic disease
15:00 – 15:15	**Cutting Edge: Oral Abstract Presentation** Caroline M. Percopo (NIAID, NIH, Bethesda, USA)	Immunomodulatory and anti-fibrotic properties of eosinophils in late stage *Schistosoma mansoni* infection
15:15 – 15:30	Achim Hoerauf (University of Bonn, Germany)	Role of IL-5 and eosinophils in limiting adult worm establishment in murine and human filariasis
15:30 – 15:45	David Abraham (Thomas Jefferson University School of Medicine, Philadelphia, USA)	Eosinophil interaction with a parasitic nematode: recruitment, killing and antigen presentation
15:45 – 16:30	**BREAK**	
SOCIAL EVENT: SALT LAKE CITY OUTING		
16:10	Buses board in front of Cliff Lodge for downtown Salt Lake City (Hardware Building)	
16:30	Buses leave for Salt Lake City	
17:15 – 22:30	Dinner at Hardware Building + Salt Lake City Sightseeing (5.25 hours)	
22:30	Buses leave Hardware Building for return to Snowbird	

PROGRAM

FRIDAY, JULY 20 — MORNING

7:00 – 8:00	**BREAKFAST (provided for symposium registrants)**	
SESSION: EOSINOPHILS AND DISEASE — PATIENTS TO ANIMAL MODELS AND BACK TO PATIENTS (DAY I)		
Asthma – Moderator: Steven Ackerman		
8:00 – 8:30	**State of the Art Presentation – Animal Models** James Lee (Mayo Clinic Arizona, Scottsdale, USA)	
8:30 – 8:45	Mark Inman (McMaster University, Hamilton, Canada)	Steroid Treatment In Mouse Models Of Chronic Exposure To Allergen
8:45 – 9:00	Douglas Robinson (National Heart and Lung Institute, London, UK)	Eosinophils and Disease: Patients to Animal Models and Back to Patients
9:00 – 9:15	**Cutting Edge: Oral Abstract Presentation** Elizabeth A. Jacobsen (Mayo Clinic Arizona, Scottsdale, USA)	Eosinophils are required for allergen-induced Th2 inflammation
Gastrointestinal Diseases – Moderator: Glenn Furuta		
9:15 – 9:45	**State of the Art Presentation – Animal Models** Marc Rothenberg (University of Cincinnati, USA)	
9:45 – 10:00	Simon Hogan (University of Cincinnati, USA)	Eosinophils and eosinophil chemokines, CCL11 and CCL24, in DSS-induced colonic injury
10:00 – 10:15	**Cutting Edge: Oral Abstract Presentation** Meiqin Wang (University of Cincinnati, USA)	Role of regulatory T cells in experimental eosinophilic esophagitis (EE)
10:15-10:45	**COFFEE BREAK**	
PRO – CON DEBATES		
10:45 – 11:30	Moderator: William Busse	
	Asthma Disease Management: Who cares about the airway because eosinophils are where it's at!	**15 min. presentations, 5 min. rebuttals** **Pro:** Charles Irvin (Vermont Lung Center, Burlington, USA) **Con:** Bob Schleimer (Northwestern University, Chicago, USA)
11:30 – 12:15	Moderator: Judah Denburg	
	Mouse eosinophils are fundamentally different than human eosinophils!	**15 min. presentations, 5 min. rebuttals** **Pro:** Carl Persson (University of Lund, Sweden) **Con:** Erwin Gelfand (National Jewish Center for Respiratory Research, Denver, USA)
12:15 – 15:30	**UNSCHEDULED (FREE) TIME** (3.25 hrs)	

PROGRAM

FRIDAY, JULY 20 — AFTERNOON

SESSION: EOSINOPHILS AND DISEASE — PATIENTS (DAY I) **Joint session with the Intermountain West Allergy Association (IWAA)**		
Asthma – Moderator: Paul O'Byrne		
15:30 – 16:00	**State of the Art Presentation – Patients** William Busse (University of Wisconsin, Madison, USA)	
16:00 – 16:15	Judah Denburg (McMaster University, Hamilton, Canada)	Hematopoietic stem cells as early determinants of health and disease: relevance to atopy and eosinophilic inflammation
16:15 – 16:30	Andrew Wardlaw (Leicester University, UK)	Role of eosinophils in asthma
Gastrointestinal Diseases – Moderator: Marc Rothenberg		
16:30 – 17:00	**State of the Art Presentation – Patients** Glenn Furuta (University of Colorado and Children's Hospital, Denver, USA)	
17:00 – 17:15	Mirna Chehade (Mount Sinai School of Medicine, New York, USA)	Allergic eosinophilic gastroenteritis with protein-losing enteropathy: Clinical features, intestinal pathology, and possible mechanisms of allergy
17:15 – 17:30	Calman Prussin (NIAID, NIH, Bethesda, USA)	Anti-IgE treatment of eosinophil-associated gastrointestinal disorders
17:30-17:45	**COFFEE BREAK**	
SIMULTANEOUS SESSIONS (17:45 – 19:15): POSTER VIEWING AND DISCUSSION I AND II		
Session I: Cell Biology and Eosinophil Studies Topics and Posters Programmed from Submitted Abstracts (Posters # 1 – 29)		
Moderators: Steven Ackerman and Christine Bandeira-Melo		
17:45 – 18:30	Poster Viewing *(Magpie Room)*	
18:30 – 19:15	Discussion Session: Presenting Author Summaries, Questions/Answers *(Ballroom I)*	
Session II: Animal Models and Patient Studies Topics and Posters Programmed from Submitted Abstracts (Posters # 30 – 64)		
Moderators: Peter Weller and Paul Foster		
17:45 – 18:30	Poster Viewing *(Magpie Room)*	
18:30 – 19:15	Discussion Session: Presenting Author Summaries, Questions/Answers *(Ballroom II)*	
SOCIAL EVENT: BBQ DINNER AT SNOWBIRD		
19:30 – 22:30	Conference center terrace tent, Cliff Lodge (level 1)	

PROGRAM

SATURDAY, JULY 21 — MORNING

7:00 – 8:00	**BREAKFAST (provided for symposium registrants)**	
SESSION: EOSINOPHILS AND DISEASE — PATIENTS TO ANIMAL MODELS AND BACK TO PATIENTS (DAY II)		
Eosinophils and Cancer – Moderator: Francesca Levi-Schaffer		
8:00 – 8:30	**State of the Art Presentation** Michael Lotze (University of Pittsburg, USA)	
8:30 – 8:45	Francesca Levi-Schaffer (The Hebrew University of Jerusalem, Israel)	Eosinophils and allergic inflammation: from angiogenesis to hypoxia and back
8:45 – 9:00	Nancy Lee (Mayo Clinic Arizona, Scottsdale, USA)	Eosinophil tissue infiltration of tumors is a ubiquitous phenomenon characteristic of both mouse tumors and human cancers
9:00 – 9:15	**Cutting Edge: Oral Abstract Presentation** Francis Davoine (University of Alberta, Edmonton, Canada)	Human eosinophils in oral squamous cancer
Inflammation and the Role(s) of Eosinophils – Moderators: Kristin Leiferman and Sanjiv Sur		
9:15 – 9:45	**State of the Art Presentation – Inflammation and the Role(s) of Eosinophils** Paul Foster (University of Newcastle, Australia)	
9:45 – 10:00	Michael Blackburn (University of Texas – Houston, USA)	Adenosine signaling and the regulation of pulmonary inflammation: Contribution of the A3 adenosine receptor and eosinophils
10:00 – 10:15	Sanjiv Sur (University of Texas – Galveston, USA)	Pollen NADPH oxidases in allergic inflammation
10:15 – 10:30	**Cutting Edge: Oral Abstract Presentation** Masahiko Kato (Gunma Prefectural Institute of Public Health and Environmental Sciences, Maebashi, Japan)	Eosinophil granule major basic protein induces apoptosis and inflammation of bronchial epithelial cells infected with respiratory syncytial virus
10:30 – 11:00	**COFFEE BREAK**	
Eosinophil-Mediated Tissue Remodeling and Fibrosis – Moderator: Seema Aceves		
11:00 – 11:30	**State of the Art Presentation** Steven Ackerman (University of Illinois at Chicago, USA)	
11:30 – 11:45	Peter Sporn (Northwestern University, Chicago, USA)	Eosinophils and airway remodeling: TGF-β-mediated lung myofibroblast differentiation and bronchial epithelial-mesenchymal transition
11:45 – 12:00	Seema Aceves (University of California, San Diego, USA)	Tissue remodeling in pediatric eosinophilic esophagitis: who has it and does therapy help?
12:00 – 12:15	**Cutting Edge: Oral Abstract Presentation** E. A. Becky Kelly (University of Wisconsin, Madison, USA)	Contribution of TNF-α to the exquisite adaptability of the eosinophil to influence inflammation and repair
12:15 – 15:00	**UNSCHEDULED (FREE) TIME** (2.75 hr)	
12:15 – 14:15	**Meeting of the IES Executive Board** *(White Pine Room)*	

PROGRAM

SATURDAY, JULY 21 — AFTERNOON

SESSION: EOSINOPHILS AND DISEASE — PATIENTS TO ANIMAL MODELS AND BACK TO PATIENTS (DAY II)		
Viral, Fungal, and Other Diseases – Moderator: Robert Schleimer		
15:00 – 15:30	**State of the Art Presentation** Helene Rosenberg (NIAID, NIH, Bethesda, USA)	
15:30 – 15:45	Hirohito Kita (Mayo Clinic Rochester, USA)	Innate and acquired immune responses to environmental fungi and their potential roles in human chronic airways disease
15:45 – 16:00	David Jacoby (Oregon Health Sciences University, Portland, USA)	Eosinophils in virus induced asthma attacks
16:00 – 16:15	**COFFEE BREAK**	
MINISYMPOSIUM: PAUL EHRLICH LECTURESHIP — DISCOVERY OF IL-5 AND THE DEVELOPMENT OF ANTI-IL-5 THERAPEUTICS		
16:15 – 16:20	Introduction of Paul Ehrlich Lecturers – Gerald Gleich, outgoing IES President	
16:20 – 16:30	***Paul Beeson – Posthumous Recognition*** Peter Weller (Harvard Medical School, Boston, USA)	The seminal contributions of Paul Beeson to eosinophil immunobiology
16:30 – 17:00	**Colin Sanderson** (Curtin University of Technology, Albany, Western Australia)	*Paul Ehrlich Lecture* The Discovery of IL-5
17:00 – 17:30	**Kiyoshi Takatsu** (The Institute of Medical Science, University of Tokyo, Japan)	*Paul Ehrlich Lecture* The Discovery of IL-5
17:30 – 17:40	Presentation of the Ehrlich Awards – Steven Ackerman, incoming IES President	
17:40 – 18:10	**State of the Art Presentation – Anti-IL-5 Therapeutics: History, Development and Clinical Trials in Asthma** Paul O'Byrne (McMaster University, Hamilton, Canada)	
18:10 – 18:40	**State of the Art Presentation – Anti-IL-5 Therapeutics: Clinical Trials in Hypereosinophilic Syndromes** Amy Klion (NIAID, NIH, Bethesda, USA)	
SOCIAL EVENT: SYMPOSIUM BANQUET AT MARKET STREET GRILLE, COTTONWOOD CANYON		
19:00	Buses board in front of Cliff Lodge for Market Street Grill	
19:10	Buses leave for Market Street Grill	
19:30	Conference Banquet **Presentation of IES Young Investigator Travel Awards** – Gerald Gleich	
22:30 – 23:00	Buses return to Snowbird	

PROGRAM

SUNDAY, JULY 22 — MORNING

7:00 – 8:00	**BREAKFAST (provided for symposium registrants)**	
SESSION: EXTRAMURAL FUNDING OF EOSINOPHIL RESEARCH–WHERE TO FIND IT/HOW TO GET IT!		
Moderator: James Lee		
8:00 – 8:30	**State of the Art Presentation: The National Institutes of Health Perspective** Michael Minnicozzi (NIAID, NIH, Bethesda, USA)	
8:30 – 8:45	Deborah Slipetz (Merck Frosst, Canada)	The Pharmaceutical Industry's Perspective
8:45 – 9:00	Leo Koenderman (Utrecht University, Netherlands)	The Investigator's Perspective (Europe)
9:00 – 9:10	**COFFEE BREAK**	
SESSION: NOVEL METHODS AND APPROACHES TO STUDIES OF EOSINOPHIL BIOLOGY AND EFFECTOR FUNCTIONS		
Moderators: Monique Capron and Klaus Matthaei		
9:10 – 9:25	Klaus Matthaei (John Curtin School of Medical Research, Canberra, Australia)	Towards reversible temporal deletion of eosinophils in vivo
9:25 – 9:40	Arne Slungaard (University of Minnesota, Minneapolis, USA)	Thiocyanate-dependent inhibition of spontaneous and agonist-induced eosinophil apoptosis and degranulation by eosinophil peroxidase (EPO): A potential physiologic role for endogenously generated HOSCN in maintaining eosinophil viability
9:40 – 9:55	**Cutting Edge: Oral Abstract Presentation** Cheryl Protheroe (Mayo Clinic Arizona, Scottsdale, USA)	Identification and improved diagnosis of eosinophilic esophagitis patients from archived clinical biopsies using a novel anti-eosinophil peroxidase monoclonal antibody
9:55 – 10:10	**Cutting Edge: Oral Abstract Presentation** Kimberly Dyer (NIAID, NIH, Bethesda, USA)	*Ex vivo* differentiation of eosinophils from the ΔDdblGATA mouse
10:10 – 10:30	**Cutting Edge: Oral Abstract Presentation** Roland Kolbeck (MedImmune Inc., Gaithersburg, USA) William Busse (University of Wisconsin, Madison, USA)	MEDI-563, is a humanized anti-human IL-5Rα antibody with enhanced effector function, and is well tolerated and induces reversible blood eosinopenia in mild asthmatics in a phase I trial, MI-CP-158
10:30 – 10:45	**COFFEE BREAK**	
SESSION: INTERNATIONAL EOSINOPHIL SOCIETY — MEETING SUMMATION, IDEAS AND PERSPECTIVES FOR THE FUTURE		
Moderators: Steven Ackerman and Jamie Lee		
10:45 – 11:15	**Basic Science Highlights and Future Perspectives** Redwan Moqbel (University of Alberta, Edmonton, Canada)	
11:15 – 11:45	**Clinical/Translational Highlights and Future Perspectives** Gerald Gleich (University of Utah, Salt Lake City, USA) William Busse (University of Wisconsin, Madison, USA)	
11:45 – 12:15	**Open Discussion** – Future Directions of the IES and Eosinophil Research	
12:15 – 12:30	**ACKNOWLEDGEMENTS AND ADJOURNMENT –** Gerald Gleich	

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – EOSINOPHIL TRAFFICKING, ACTIVATION/INHIBITION, AND SIGNAL TRANSDUCTION

Leo Koenderman, Henk Honing and Laurien Ulfman

Department of Respiratory Medicine, University Medical Center, Utrecht, The Netherlands.

Background: Eosinophils belong to the most cytotoxic cells in the body, which are controlled by a multitude of receptors. These receptors are involved in several cellular responses mediating differentiation/priming, adhesion/homing, cytotoxicity and apoptosis

Objectives: To review recent advances in eosinophil signaling in relation to these cellular responses

Methods: Signal transduction in primary eosinophils is complicated by the fact that they are rare white blood cells, which are difficult to manipulate by molecular techniques. However, recent advances in fluorescence based methodology makes it possible to study signal response coupling in detail. Application of new pharmacological inhibitors and novel methodology utilizing cell permeable compounds (proteins/RNAi) allows the definition of complex control mechanisms in human eosinophils.

Results: Several signaling pathways have been identified in receptor response coupling in human eosinophils: PI-3/PKB and SRC-kinases in differentiation and adhesion mediated by cytokine receptors, changes in $[Ca^{2+}]_i$ and activation of small molecular G-proteins induced by chemokine receptors, and MAP-kinases in cellular priming. Gene-array and proteomic studies have identified new targets that are likely involved in signal-response coupling in human eosinophils. Particular interest is recently given to the fact to the aforementioned pathways interact and novel methodology is developed to study these complex pathways.

Conclusions: Studies in signal response coupling in eosinophils have identified novel intracellular pathways that show that eosinophils are controlled by unique signals. These signals will be reviewed in the context of eosinophils responses that can be targets for future therapy.

INVITED SPEAKER ABSTRACTS

PIN1 CONTROLS PULMONARY EOSINOPHILIC INFLAMMATION

Stephane Esnault, Zhong-Jian Shen, Louis A. Rosenthal, Renee J. Szakaly, Ronald L. Sorkness and James S. Malter

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The infiltration, accumulation and degranulation of eosinophils in the lung represent a hallmark of active asthma. Eosinophil activation *in vivo* or *in vitro* triggers the secretion of the anti-apoptotic cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF). We recently showed that Pin1 regulates the production of GM-CSF at the post-transcriptional level *in vitro* in eosinophils and T lymphocytes. Here, we confirm that splenocytes from Pin1 KO mice are unable to stabilize or accumulate GM-CSF mRNA after activation. Treatment with juglone, a Pin1 inhibitor had no additional effect on cytokine secretion from KO cells. Therefore, we sensitized Brown Norway rats with ragweed pollen extract prior to aerosol challenge. Rats treated with juglone showed significantly reduced BAL eosinophils compared to untreated sensitized and challenged controls. GM-CSF and major basic protein mRNAs were also significantly reduced. These data suggest that in vivo Pin1 blockade attenuates GM-CSF production and eosinophilic allergic inflammation.

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INVITED SPEAKER ABSTRACTS

TWO DIFFERENT FACES OF GSK-3: REGULATION OF EOSINOPHIL MATURATION AND SURVIVAL

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Interleukin (IL)-5 is a hematopoietic cytokine able to regulate differentiation, survival and effector functions of eosinophils, however the molecular mechanisms underlying these events have remained largely elusive. Beyond its role in glycogen metabolism, glycogen synthase kinase-3 (GSK-3) acts as a downstream regulatory switch that determines the output of numerous signalling pathways initiated by diverse stimuli. Unlike most protein kinases, GSK-3 is constitutively active under resting conditions and undergoes inhibitory phosphorylation after cellular stimulation. Using human peripheral blood eosinophils we found that IL-5 induces phosphorylation and thus inactivation of GSK-3, while conversely cytokine withdrawal results in GSK-3 activation.

In human peripheral blood eosinophils IL-5 withdrawal results in activation of a mitochondria-dependent apoptotic program, independently of Fas receptor activation. Importantly, abrogation of GSK-3 activity results in enhanced eosinophil survival in the absence of cytokine. β-catenin, a direct GSK-3 substrate, was found to be present in the nucleus of IL-5 stimulated eosinophils, but was translocated to the plasma membrane in the absence of cytokine and in a GSK-3 dependent manner. These data suggest a novel molecular mechanism by which IL-5 inhibits the constitutive apoptotic program active in mature eosinophils. Upregulation of β-catenin target genes may be responsible for maintaining eosinophil viability in the presence of IL-5.

We have previously demonstrated that modulation of the phosphatidylinositol 3-kinase (PI3K) signalling module can regulate lineage choice decisions during granulopoiesis. Since activation of PI3K results in inhibition of GSK-3 we analysed the role of GSK-3 in regulating eosinophil development. Ectopic expression of constitutively active GSK-3 in CD34+ cord blood derived progenitors resulted in enhanced eosinophil differentiation. GSK-3 was also found to regulate phosphorylation of C/EBPα (Thr222/226) thereby inhibiting its transcriptional activity. Introduction of a non-phosphorylatable C/EBPα mutant into CD34+ progenitors resulted in inhibition of eosinophil differentiation. These data provide evidence for a role for GSK-3 in regulating eosinophil production through inhibition of C/EBPα mediated transcription.

Taken together these data demonstrate a complex role for GSK-3 in the regulation of eosinophil numbers, affecting both differentiation of hematopoietic progenitor cells as well as survival of mature eosinophils. Modulation of GSK-3 activity by specific pharmacological inhibitors may thus hold promise for the development of novel strategies to modulate eosinophil numbers *in vivo*.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – AIRWAY EOSINOPHILS: ALLERGIC INFLAMMATION RECRUITED PROFESSIONAL ANTIGEN-PRESENTING CELLS

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Background: Eosinophils, not normally common within airways tissues, are however typically recruited during allergic inflammatory diseases of the nares and lungs. Although recruited airway eosinophils, as "end-stage" effector cells, may contribute locally to airway inflammation, the capacities of recruited, airway "inflammatory" eosinophils to migrate from within the airways and to function as fully capable, immunomodulatory antigen-presenting cells (APCs) have been uncertain.

Objectives: We investigated whether airway eosinophils can migrate from the airways lumina to lymphoid organs and function as professional APCs to elicit antigen-naïve T cell responses *in vivo*.

Methods: Eosinophils were isolated free of other APCs from IL-5 or IL-5/GFP transgenic (tg) mice. Purified eosinophils were cultured with GM-CSF and incubated for 1 hr with antigen (OVA or OVA-coupled to blue fluorescent microbeads) prior to being instilled intratracheally into mice that were recipients of adoptive transfers of OVA antigen-specific $CD4^+$ T cells from OVA TCR tg mice. Proliferation (BrdU incorporation), activation (CD69 expression) and cytokine (IL-4, IFN-γ) production by OVA-specific $CD4^+$ T cells were assessed in paratracheal lymph nodes (pLN). Interactions amongst green GFP-tg eosinophils bearing blue-fluorescent OVA beads with red fluorochrome-labeled T cells were assessed in pLN. Migrations of airways eosinophils to systemic lymph sites were studied.

Results: Eosinophils expressed MHC Class II and costimulatory proteins, CD40, CD80 and CD86. OVA-pulsed eosinophils, instilled intratracheally into mice that received adoptive transfers of OVA-specific $CD4^+$ T cells, elicited activation, proliferation and IL-4, but not IFN-γ, cytokine production by OVA-specific $CD4^+$ T cells in pLNs. Cognate physical interactions of green-GFP eosinophils bearing blue-OVA with naïve red-labeled OVA-specific $CD4^+$ T cells were demonstrable in pLNs. Moreover, airway eosinophils migrated not only to regional pLNs but also to splenic and thymic systemic lymphoid sites.

Conclusions: Thus, recruited, luminal airway eosinophils are distinct allergic "inflammatory" full-function APCs. Recruited airways eosinophils can provide additional APC functions, over and above other normal lung resident lung APCs. Roles for eosinophils in often chronic, ongoing allergic airways inflammation can include their capacities as "allergic inflammatory" APCs to present airway-encountered allergens to modulate T cell-mediated immune responses.

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INVITED SPEAKER ABSTRACTS

GALECTIN-3 AND EOSINOPHIL TRAFFICKING

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Background: The mobilization and trafficking of eosinophils to sites of allergic inflammation is a complex process involving sequential engagement of multiple adhesion molecules, cytokines and chemokines. Galectin-3 (Gal-3) is a member of a family of b-galactoside-binding animal lectins. While several functions have been ascribed in vitro, recent studies have shown that Gal-3 can differentially modulate eosinophil recruitment and airway allergic inflammation in mice. However, Gal-3 lacks a transmembrane domain and therefore its ability to be expressed on the cell surface and function as an adhesion molecule to support eosinophil trafficking is not well understood.

Objective: To examine if eosinophils and endothelial cells express Gal-3 and support eosinophil trafficking (rolling and adhesion) under conditions of flow.

Methods: Eosinophils and endothelial cells were examined for their ability to express Gal-3 on the cell surface by flow cytometry and confocal microscopy. Using a parallel plate flow chamber assay and function blocking antibodies, the ability of Gal-3 to support eosinophil rolling and adhesion on immobilized VCAM, Gal-3 or IL-1b stimulated endothelial cells was determined.

Results: Gal-3 was found to be expressed on the cell surface of eosinophils as well as IL-1β stimulated endothelial cells. Interestingly, Gal-3 was found to be co-locally expressed with a4 integrin on eosinophils. This was supported by the demonstration of direct binding of recombinant α4β1 to Gal-3. Under conditions of shear stress, significantly increased rolling and firm adhesion of eosinophils was observed on immobilized Gal-3 as well as VCAM-1. Inhibition studies with function blocking mAbs as well as lactose demonstrated that a4 integrin supports eosinophil rolling and adhesion on immobilized Gal-3, and that eosinophil-expressed Gal-3 interacts with immobilized Gal-3 through the carbohydrate recognition domain of Gal-3, a finding that was further confirmed using IL-1b stimulated endothelial cells under conditions of flow. Furthermore, pretreatment of eosinophils with soluble Gal-3 resulted in inhibition of rolling and adhesion on Gal-3 as well as VCAM-1. Overall, these studies support the findings that while immobilized Gal-3 can participate and promote the trafficking of eosinophils to sites of inflammation, exposure to soluble Gal-3 can result in the inhibition of eosinophil trafficking.

Conclusion: Our studies suggest that although Gal-3 lacks a transmembrane domain, it can function as a cell surface adhesion molecule potentially through binding to adhesion receptors such as a4b1 to support eosinophil rolling and adhesion under conditions of flow. However, exposure to soluble Gal-3 may also result in inhibition of eosinophil trafficking.

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INVITED SPEAKER ABSTRACTS

PLATELETS AND EOSINOPHILS: A CLOSE CONTACT RELATIONSHIP

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It is now widely accepted that the role of platelets is not limited to their function in haemostasis and thrombosis. Accumulating evidence suggests they are implicated in the inflammatory response characteristic of many different disorders, including asthma, rheumatoid arthritis, atherosclerosis and even tumour metastasis. In allergic diseases, specifically in asthma, allergic rhinitis and dermatitis, platelets have been shown to actively participate in the characteristic recruitment of eosinophils. This role as "chaperones" has been exemplified by studies showing the existence of a close contact relationship between platelets and eosinophils, thus suggesting the actual adhesion is more important than any platelet-released mediators. In addition, platelets are known to express both high-affinity and low-affinity IgE receptors, but the proportion of platelets bearing these receptors increases significantly in allergic individuals and animals. Taken together, the data available to date clearly suggests that platelets have a prominent function in allergy, acting as inflammatory cells involved in the recruitment of eosinophils. Thus, platelets have the potential to provide much-needed novel therapeutic targets in areas where existing therapies are far from optimal.

INVITED SPEAKER ABSTRACTS

NON-HEMATOPOIETIC NADPH OXIDASE REGULATION OF LUNG EOSINOPHILIA AND AIRWAY HYPERRESPONSIVENESS IN EXPERIMENTALLY-INDUCED ASTHMA

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Background: Pulmonary eosinophilia is one of the most consistent hallmarks of asthma. Infiltration of eosinophils into the lung in experimental asthma is dependent on the adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells. Ligation of VCAM-1 activates endothelial cell NADPH oxidase which is required for VCAM-1-dependent leukocyte migration in vitro.

Objective: To determine whether endothelial-derived NADPH oxidase modulates VCAM-1-dependent eosinophil recruitment in vivo.

Methods: To examine whether endothelial-derived NADPH oxidase modulates eosinophil recruitment in vivo, mice deficient in NADPH oxidase (CYBB mice) were irradiated and received wild type hematopoietic cells to generate chimeric CYBB mice. These mice were sensitized and challenged with OVA since eosinophil recruitment is largely dependent on binding to VCAM-1. Inflammation and airway hyperresponsiveness was examined.

Results: In response to OVA challenge, the chimeric CYBB mice had increased numbers of eosinophils bound to the luminal surface of the endothelium as well as reduced eosinophilia in the lung tissue and bronchoalveolar lavage. This occurred independent of changes in VCAM-1 expression, cytokine/chemokine levels (IL-5, IL-10, IL-13, IFNγ, or eotaxin), or numbers of T cells, neutrophils or mononuclear cells in the lavage fluids or lung tissue of OVA-challenged mice. Importantly, the OVA-challenged chimeric CYBB mice had reduced airway hyperresponsiveness (AHR). The AHR in OVA-challenged chimeric CYBB mice was restored by bypassing the endothelium with intratracheal administration of eosinophils.

Conclusions: These data suggest that VCAM-1 induction of NADPH oxidase in the endothelium is necessary for the eosinophil recruitment during allergic inflammation. Moreover, these studies provide a basis for targeting VCAM-1-dependent signaling pathways in asthma therapies.

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INVITED SPEAKER ABSTRACTS

LOCALIZATION AND CHARACTERIZATION OF GLYCAN LIGANDS FOR SIGLEC-F, THE MURINE PARALOG OF THE EOSINOPHIL INHIBITORY RECEPTOR SIGLEC-8.

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Background: Siglec-F and Siglec-8 are functional paralog inhibitory receptors on mouse and human eosinophils respectively. Both Siglecs preferentially recognize the unique ligand NeuAcα2–3(6-*O*-sulfo)Galβ1–4[Fucα1–3]GlcNAc (6'-sulfated sialyl Lewis X or 6'-su-sLeX), but the tissue distribution of this endogenous ligand is unknown.

Objectives: To explore tissue expression of 6'-su-sLeX and the unique sialyltransferases and sulfotransferase required for its expression in murine pulmonary tissues.

Methods: Distribution of ligands on selected C57BL/6 mouse lung tissues (wild type, OVA sensitized and challenged, and IL-13 lung transgenics) was studied via histochemistry using Siglec-F-Ig fusion proteins and Ig controls. The sialic acid binding dependency of Siglec-F-Ig fusion protein was also confirmed by pre-treating tissue sections with neuraminidase. Patterns of Siglec-F-Ig protein binding were compared to binding patterns of plant lectins specific for α2,3 or α2,6-linked sialic acids (Maakia amurensis, [MAA] and Sambucus nigra, [SNA], respectively). RT-PCR was used to detect the sialyltransferase ST3Gal IV, and the sulfotransferase KSGal6ST, needed for 6'-su-sLeX synthesis.

Results: Using histochemistry, Siglec-F-Ig fusion protein (but not controls including Siglec-10-Ig fusion protein and Ig control) selectively bound to normal airways epithelium and type II alveolar epithelial cells. This binding pattern was identical to that seen with MAA, while SNA staining was non-epithelial and more diffusely parenchymal. After neuraminidase pretreatment of the tissue sections, Siglec-F-Ig fusion protein, MAA and SNA all failed to bind, indicating their sialic acid binding dependence. Patterns of Siglec-F-Ig protein binding and MAA binding were similar in normal and Th2-inflamed lungs, suggesting that the constitutive expression of these glycans on these cells is not clearly altered under these conditions. Siglec-F-Ig fusion protein binding was inhibited by pretreatment of mouse lung tissue sections with MAA lectin, but not SNA lectin, suggesting that the epithelial glycan ligand recognized by Siglec-F-Ig fusion protein contains α2,3-linked sialic acids. Quantitative RT-PCR detected both ST3Gal IV and KSGal6ST in whole mouse lung, but only the latter was increased in expression in Th2-inflamed whole mouse lung.

Conclusions: α2,3-linked sialic acid-containing Siglec-F ligands, and enzymes required for 6'-su-sLeX synthesis, are constitutively expressed in mouse lung. Th2 inflammation is associated with increased KSGal6ST expression. Eosinophils entering the lung may have their survival shortened by encountering these endogenous Siglec-8 sialoside ligands.

This project was funded by grant #AI41472 from the National Institutes of Health; Dr. Bochner also received support from Johns Hopkins as a Cosner Scholar in Translational Research.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – EFFECTOR FUNCTIONS OF EOSINOPHILS

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Eosinophils effector functions are the subject of a considerable literature, and the National Library lists hundreds of articles on this subject. Thus, rather than having a dearth of data, one is confronted with a surfeit! In this review, I will consider the stages in the historical life of the eosinophil and our concepts of its functions. In addition, I will discuss the effectors used by the eosinophil and relate this information to the pathophysiology of bronchial asthma.

Ehrlich regarded the eosinophil as a storage cell based on the prominence of its granules. Early in the 20th century, histological analyses of guinea pig lungs following anaphylactic reactions showed a prominent eosinophil infiltration, and this finding suggested that the eosinophil altered anaphylaxis. The concept of the eosinophil as an anti-inflammatory cell regulating anaphylaxis and mast cells mediators reigned unchallenged during most of the 20th century. Observations that eosinophils diminished the reactivity of histamine and degraded mediators of anaphylaxis supported this concept of eosinophil function. Analyses of eosinophil granule proteins and their functions ushered in the concept that the eosinophil is a cell mediating toxicity to helminths and tissues. However, recent findings show that the eosinophil produces numerous cytokines and chemokines (with a host of biological effects). Therefore, our concepts of the critical functions of eosinophils are in a state of flux. Whereas considerable experimental data support a role for the eosinophil as a mediator of tissue damage, the new information on the plethora of molecules produced by eosinophils opens new conceptual vistas.

Eosinophils likely have a role in normal growth and health, including reproduction and immune regulation, especially in protection against helminths and even against malignant tumors. However, in this presentation, I will focus on bronchial asthma. In asthma, recent information both from animal models and from patients implicates the eosinophil as a mediator of tissue damage and as a marker of disease activity. Numerous reports suggest that the eosinophil is a major cause of bronchial hyperresponsiveness, but animal models have yielded conflicting results. In contrast, considerable information points to an important role for the eosinophil in remodeling based on its production of transforming growth factor beta and the ability of granule proteins to stimulate production of profibrotic molecules by respiratory epithelium. Eosinophils modify nerve function, and eosinophil mediators block muscarinic M2 receptors and increase the sensitivity of pulmonary afferent nerves. Evidence also suggests that eosinophils, possibly via production of trophic molecules, such as nerve growth factor, exert reparative effects on nerves. All of these activities may be important in asthma.

We have reached a new plateau in investigations of eosinophils. Previously we were content with observations in vitro and their implications for disease. Presently we realize that we must verify our concepts in animal models or in patients. Investigations of animal models, especially the mouse, offer enormous opportunities, whereas in patients our tools are limited. The recent studies of antibodies to interleukin-5 provide the first opportunities for tests in human disease. We need new pharmaceutical tools to alter eosinophils in patients and test the role of the eosinophil in disease.

INVITED SPEAKER ABSTRACTS

EXTRACELLULAR TRAPS GENERATED BY GRANULOCYTES – EFFECTOR FUNCTION OF VIABLE CELLS OR RESULT OF A NEW FORM OF CELL DEATH?

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Background: The phenomenon of DNA-containing extracellular traps, which also contain granule components, has been described in association with neutrophil activation in vitro. It is believed that these traps play an important role in anti-bacterial defense mechanism. Recently, the formation of neutrophil extracellular traps (NETs) has been associated with a new, non-apoptotic form of neutrophil cell death.

Objectives: It was the aim of this study to evaluate whether eosinophils are able to form extracellular traps (EETs) and whether cell death was required to generate such structures.

Methods: Purified eosinophils were stimulated with different agonists. EETs were analyzed by confocal microscopy and several methods to analyze extracellular DNA in a qualitative and quantitative manner. Cell death and apoptosis was analyzed by commonly used techniques.

Results: Eosinophils were able to generate extracellular traps, which contained DNA and basic granule proteins, such as eosinophil cationic protein (ECP) and major basic protein (MBP). No cytosolic or membrane proteins were identified as components of EETs. Formation of EETs was not associated with eosinophil death or apoptosis. Similar results were observed in neutrophils.

Conclusions: Upon activation, granulocytes are able to generate extracellular traps, which contain DNA and granule proteins. Cell death is not a requisite for extracellular trap formation by granulocytes. The functional role of EETs remains unclear.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND PARASYMPATHETIC NERVE FUNCTION IN LUNGS

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Parasympathetic nerves recruit eosinophils by secreting chemotactic factors that are increased during lung inflammation as with antigen challenge. Parasympathetic nerves also express ICAM and VCAM, which are recognized by counterligands on eosinophils. Both chemotactic factors and adhesion molecules are important physiologically since eosinophils are clustered around parasympathetic nerves in humans with asthma. Similarly, eosinophils surround airway nerves in animal models of asthma, where they mediate airway hyperreactivity. Activated eosinophils release major basic protein, an endogenous antagonist for inhibitory M2 muscarinic receptors on the airway nerves. Blocking neuronal M2 muscarinic receptors increases acetylcholine release and and increases vagally-induced bronchoconstriction. Interfering with either chemotactic factors or adhesion molecules protects M2 receptor function and prevents airway hyperreactivity by preventing eosinophil clustering around nerves, while having little effect on eosinophil migration to the whole lung. However, why parasympathetic nerves would need to recruit eosinophils is still unclear.

One clue to the interaction of eosinophils and nerves comes from studies in ozone exposed guinea pigs. Exposure to ozone induces airway hyperreactivity lasting at least three days. Ozone also induces eosinophil hematopoesis and eosinophil populations in lung fluctuate over this time period, so that by three days post ozone more than 80% of eosinophils in the lungs are new (as assessed by BRDU labeling). The role of these new eosinophils is uncertain but if they are prevented from entering the lungs with AbVLA-4 or depleted with AbIL-5, ozone induced hyperreactivity is significantly worse at this time point than in the presence of eosinophils. Eosinophils release neurotrophic factors that may be involved in a repair process, but since antibodies to nerve growth factor prevent ozone induced hyperreactivity three days post ozone the protective/repair/exacerbating roles of eosinophils and neurotrophins is still unclear. However, atopy changes the role of eosinophils three days post ozone. In atopic guinea pigs, eosinophils have only a deleterious role and depletion is protective at all time points after a single ozone exposure.

While it appears that eosinophils can alter nerve activity via release of major basic protein and possibly also by release of neurotrophins, we now have evidence that nerves may affect eosinophil function. Eosinophils express muscarinic receptors, and while the function of these receptors is not known, administration of atropine before antigen challenge increased subsequent eosinophil degranulation and increased airway hyperreactivity. Thus, parasympathetic nerves may inhibit eosinophil function via muscarinic receptors. It therefore appears there is a two way interaction between eosinophils and airway nerves. The role of this interaction in disease and repair remains to be explored.

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INVITED SPEAKER ABSTRACTS

EOSINOPHIL REGULATION OF TH2 CYTOKINE SECRETION FROM CD4+ T-CELLS IS DEPENDENT ON ICAM-1

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Background: Eosinophils (EOS) and T-cells are prominent components of the allergic inflammatory milieu. We have previously shown that human eosinophils are capable of enhancing the activation of CD4+ T-cells stimulated by Staphylococcal enterotoxin B (SEB) as measured by cytokine secretion and proliferation.

Objectives: We sought to determine the mechanism for the EOS mediated regulation of CD4+ T-cell cytokine secretion.

Methods: EOS and CD4+ T-cells were purified by negative selection from human peripheral blood. Cells were cultured with SEB (3μg/mL) for 48 hours and supernatants were collected for ELISA of IL-13 and IFN-γ protein levels. Transwell 0.4μm semi-permeable membranes were utilized for separation of EOS and SEB stimulated CD4+ T-cells in co-culture. Flow cytometry was performed to examine surface expression of intercellular adhesion molecule (ICAM)-1 on EOS. Inhibition of ICAM-1 was performed with the introduction of an anti-ICAM-1 neutralizing monoclonal antibody at 1μg/mL in the co-culture of EOS and SEB stimulated CD4+ T-cells.

Results: The enhanced secretion of IL-13 and IFN-γ upon co-culture of EOS with SEB stimulated CD4+ T-cells was abolished by introduction of the Transwell membrane suggesting that cell contact is required. Flow cytometry revealed that ICAM-1 expression on EOS is induced upon co-culture with SEB stimulated CD4+ T-cells. The use of the neutralizing anti-ICAM-1 monoclonal antibody inhibited the ability of EOS to enhance SEB stimulated CD4+ T-cell cytokine secretion of IL-13, while a control IgG antibody had no effect.

Conclusions: EOS regulation of the CD4+ T-cell secretion of IL-13 is mediated by cell contact via integrin interactions involving ICAM-1.

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INVITED SPEAKER ABSTRACTS

EOSINOPHIL SUPEROXIDE RELEASE AND AIRWAY HYPERRESPONSIVENESS ARE NOT DEPENDENT ON RAC2 GTPASE

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Background: Superoxide production from eosinophils undergoing respiratory burst correlates with asthma severity, and is thought to contribute to allergic symptoms by causing edema and tissue inflammation. Superoxide generation is dependent on activation of NADPH oxidase by a GTP-bound Rho-related guanosine triphosphatase (GTPase), Rac1 or its homolog Rac2. While neutrophils express mainly Rac2, and Rac2 is the dominant protein that activates NADPH oxidase, it is not known whether Rac1 or Rac2 preferentially activates the oxidase in eosinophils. Our earlier studies indicated that Rac2 is required for eotaxin-2-induced chemotaxis in eosinophils, demonstrating functional consequences in eosinophils. Here we determined whether Rac2 is a central regulator of mediator release and immune function in eosinophils.

Objectives: To determine whether Rac2 regulates the production of superoxide release from eosinophils, and whether Rac2 mediates inflammation and airway hyperresponsiveness (AHR).

Methods: We isolated splenic eosinophils from CD2-IL-5 transgenic mice (WT) and Rac2-deficient mice bred against the CD2-IL-5 transgenic background (Rac2 KO/IL-5 Tg), and compared their ability to release superoxide in response to phorbol myristate acetate (PMA). To determine allergic inflammatory responses, we subjected mice to intraperitoneal sensitization with ovalbumin (OVA) and alum followed by intranasal OVA challenge, or intranasal sensitization to cockroach allergens, and compared the responses of WT C57Bl/6 mice with Rac2 KO mice. Responses were determined by bronchoalveolar lavage cell counts and Penh measurements for AHR.

Results: Surprisingly, whole spleen and MACS-purified splenic eosinophils from Rac2 KO/IL-5 Tg mice showed similar levels of superoxide release to those of WT mice. This was in contrast to Rac2 KO neutrophils, which exhibit a deficiency (~30% of control values) in superoxide release. In both models of airway inflammation and AHR, Rac2 KO mice developed eosinophilia in BAL samples and hyperresponsiveness that was similar to control wild type mice.

Conclusions: These findings suggest that gene deletion of Rac2 does not affect eosinophil superoxide release or its transmigration into the airways. We concluded that, unlike neutrophils, eosinophil-related inflammatory processes occur largely independently of Rac2. This is in direct contrast to the model of neutrophil-mediated acute lung injury in Rac2 KO mice, in which there is significantly less airway inflammation and injury. We propose that Rac1 may instead be a critical regulator of eosinophil activation.

This project was funded by grants from the Canadian Institutes of Health Research and Canada Foundation for Innovation.

INVITED SPEAKER ABSTRACTS

POST-TRANSLATIONAL TYROSINE NITRATION OF EOSINOPHIL GRANULE TOXINS MEDIATES IMPROVED KILLING OF *TRYPANOSOMA CRUZI*

Martina Ulrich[1*], Alina Petre[5*], Nikolay Youhnovski[5], Franziska Prömm[1], Charles Soukou[6], Markus Schirle[2], Michael Schumm[3], Ralph S. Pero[7], Alfred Doyle[7], James J. Lee[7], James Checkel[8], Mary Dinauer[9], Nethaji Thiyagarajan[10] K. Ravi Acharya[10], Peter Schmid[11], Felix Ratjen[12], Günter A. Schaub[5], Heinz Schwarz[4], Michael Przybylski[5] and Gerd Döring[1]

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Background: Eosinophils contain high concentrations of cationic protein toxins thought to be essential in host defence against parasites.

Objectives: To investigate whether post-translational tyrosine nitration of eosinophil granule toxins mediates improved killing of *T.cruzi* .

Methods and Results: Studies in transgenic and knock out mice revealed, that tyrosine nitration of granule-stored toxins is mediated by EPO. High resolution affinity-mass spectrometry identified specific single nitration sites at Tyr-349 in EPO and Tyr-33 in ECP. Structure modelling suggested that the nitro-Tyr residues in toxins are surface exposed. Binding assays suggested that nitration facilitates synergistic actions in host defence. Nitrotyrosine-positive eosinophil toxins induced a significantly higher level of killing of the parasite *T.cruzi* relative to non-nitrated toxins.

Conclusions: These results provide evidence that the post-translational tyrosine nitration of granule toxins is required for eosinophil effector function.

* Martina Ulrich and Alina Petre contributed equally to this work

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – EOSINOPHILS AS REGULATORS OF PHYSIOLOGICAL HOMEOSTASIS IN HOST-PARASITE INTERACTIONS

Monique Capron and Emmanuel Hermann

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Background : Eosinophils have been implicated in the physiopathology of several helminth infections, mainly due to their increased numbers in the blood of infected patients. However, their precise role in immune defense and in pathology is still a matter of debate.

Objectives: The objectives of this review are therefore to summarize data from the literature about the various factors involved in eosinophil tissue mobilisation as well as their interactions with other cell populations, and to illustrate the pleiotropic functions of eosinophils in host-parasite interactions.

Results: Several questions will be addressed: the role of eosinophils in the physiopathology of helminth infections; the nature of cellular and molecular factors involved in eosinophil tissue recruitment; the influence of eosinophils in the development of Th2 responses; their respective participation in innate and adaptive responses towards parasites; the role of eosinophils in tissue damage and/or in tissue remodeling; and finally their potential role in protozoan infections.

Conclusions: Whereas several arguments have rather proposed a role for eosinophils in the effector phase of adaptive immune responses, recent experimental evidence suggest that eosinophils can also be considered as a link between innate and adaptive immunity.

INVITED SPEAKER ABSTRACTS

PARASITE-ENCODED IL-5 RECEPTOR BINDING PROTEIN ILLUSTRATES A STRATEGY USED BY HELMINTH PARASITES FOR IMMUNE EVASION

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Background: Peripheral blood eosinophilia occurs very early following infection with tissue invasive helminth parasites, earlier than could be accounted for by a parasite antigen induced adaptive T cell-mediated immune response.

Objectives: To identify and characterize a filarial parasite-encoded molecule with IL-5-like activity.

Methods: A phage display *Brugia malayi* infective larval (L3) expression library was panned against the soluble human IL5R. The molecule identified was characterized at the molecule level and expressed as a recombinant full-length protein (rBmIL5Rbp). Binding to human IL-5R was assessed using surface plasmon resonance. The activity of rBmIL5Rb on human eosinophils was determined in survival assays, by surface binding characteristics, and by intracellular flow cytometry for STAT-phosphorylation.

Results: A *Brugia malayi*-encoded IL5Rbp was identified that has no known homologues in mammalian genomes. This 19kDa protein termed BmILRbp, when expressed in recombinant form and purified was shown to bind to the human IL5R in the micromolar range by plasmon surface resonance. Antibodies raised to the recombinant protein showed specific staining in the cuticle and in the basal lamina of L3 by immunoelectron microscopy and reacted with the excretory/secretory products of the L3. rBmIL5Rbp was unable to induce STAT-phosphorylation in human eosinophils nor did it prolong eosinophil survival. Rather, it inhibited the ability of human IL-5 to prolong eosinophil survival. Indeed, two distinct peptides (20 mers) derived from the sequence of the BmIL5Rbp were also able to inhibit human IL-5 from binding to the surface of human eosinophils.

Conclusions: A parasite-encoded and secreted molecule from filarial parasites binds human IL-5R on eosinophils and antagonizes the activity of human IL-5. These data suggest that helminth parasites have evolved complex (and often unique) strategies for subverting the human immune response.

INVITED SPEAKER ABSTRACTS

ECP AND EPX IN PARASITIC DISEASE

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Background: Our recent findings show that a common single nucleotide polymorphism (SNP) in the ECP gene dramatically changes the function of the protein from a potent cytotoxic molecule to a non-cytotoxic molecule. Another SNP in the 3'UTR region of the gene seems to regulate the content (production) of the protein. Moreover we found clear relationships between the presence of the cytotoxic ECP and the expression of allergic symptoms such as asthma. Allergy and asthma are uncommon diseases in populations living in tropical countries with endemic exposure to parasites and helminths in spite of the fact that such exposure mostly mounts an excessive tissue and blood eosinophilia.

Objectives: The objectives of our studies are to investigate the frequencies of common SNPs in the ECP and EPX genes in African populations endemically exposed to various types of parasites and helminths and to investigate the biological consequences of such SNPs.

Results: In my presentation I will show that the frequencies of ECP and EPX SNPs vary greatly in the African populations, but also show that the occurrence of SNPs and mutations not normally found in the Scandinavian population are common. I will also show some clinical consequences of such genetic differences of which the most conspicuous finding so far is the predominance of the C-allele of the ECP434(G>C) polymorphism (which gives rise to a non-cytotoxic protein) in the schistosoma endemic areas as opposed to the predominance of the G-allele in most other populations. The reason for this is unclear, but could be related to the higher prevalence of disabling liver disease among subjects carrying the G-allele. Finally I will present some novel results on the study of the biological activities of different allele products of ECP purified from large cohorts of genotyped healthy subjects.

Conclusions: The role of eosinophils in parasitic disease is still not clear. In some diseases eosinophils may act protective, but in others the activity of the eosinophils may be turned against the body. The outcome may be determined by genetic factors such as alterations of the availability and function of two of the major eosinophil granule proteins, ECP and EPX.

INVITED SPEAKER ABSTRACTS

ROLE OF IL-5 AND EOSINOPHILS IN LIMITING ADULT WORM ESTABLISHMENT IN MURINE AND HUMAN FILARIASIS

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Background: While eosinophils (EOS) are a hallmark of filarial infection, there are few data on the role EOS play in the limitation of infection establishment, i.e. the development of adult worms from infective larvae (L3). Clearly, greater than 90% of incoming L3 are destroyed in the human body before development into adults.

Objectives: To provide evidence for a role of EOS

i) in murine filariasis caused by *Litomosoides sigmodontis*, by the use of KO mice

ii) in onchocerciasis (river blindness) caused by *Onchocerca volvulus*, by assessment of the role of IL-5 responses and EOS in re-infection rates after different therapies.

Methods: Mice genetically deficient for IL-5, IL-5 and IFN-γ, MBP and EPO were used. The development of adult worms following primary infection was monitored, as was the EOS attack rate on incoming L3 following vaccination.

For onchocerciasis, we made use of the fact that two treatments now exist: microfilaricidal ivermectin, and a new treatment with doxycycline, which targets endosymbiotic *Wolbachia* bacteria in filariae and is macrofilaricidal. Since adult worms act in an immunosuppressive manner, it was hypothesized that after ivermectin immunosuppresion is still maintained, in contrast to the macrofilaricidal therapy, with consequences for new infections.

Results: IL-5 KO mice showed highly elevated numbers of adult worms. IL-5 KO also led to reduced neutrophils at the infection site, which are the first-line cells to form inflammatory nodules around the worms. In line with the finding that neutrophils are also reduced in the absence of IFN-γ, mice deficient for both IL-5 and IFN-γ showed more elevated worm numbers, suggesting additive effects between IFN- γ and IL-5. EOS were also essential for reduced adult worm loads following vaccination with irradiated L3, and they acted via antibody–mediated release of granule proteins such as major basic protein (MBP). Accordingly, mice MBP or EPO KO mice displayed elevated worm loads, demonstrating that eosinophil granule contents are essential for adult worm containment.

In human onchocerciasis, levels of IL-5 in PBMC as well as locally in onchocercomas increased significantly more after macrofilaricidal compared to microfilaricidal treatment, in keeping with the hypothesis that macrofilaricidal therapy removes the source of immunosuppressive molecules. Interestingly, patients treated with the macrofilaricidal therapy displayed significantly less new onchocercomas after 14 months than those treated with the microfilaricidal therapy.

Conclusions: Our recent data from murine and human filariasis attribute an important role for EOS in the limitation of worm establishment from incoming L3.

This project was funded by the German Research Fund (DFG), the European Community (ICA4-2002-10051), the German Academic Exchange Service (DAAD), and the VW foundation

INVITED SPEAKER ABSTRACTS

EOSINOPHIL INTERACTION WITH A PARASITIC NEMATODE: RECRUITMENT, KILLING AND ANTIGEN PRESENTATION.

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Background: Protective immunity in mice to the nematode parasite *Strongyloides stercoralis* has been shown to be dependent on eosinophils. Eosinophils function as killer cells in the innate immune response and also support the development of the protective antibody-dependent adaptive immune response.

Objectives: The goals of these studies are to determine: **(1)** how eosinophils are recruited to the parasite; **(2)** the molecular mechanism used by eosinophils to kill the worm and **(3)** if eosinophils can function as antigen presenting cells to induce adaptive immunity to *S. stercoralis.*

Methods: (1) Experiments were performed using transwell plates to determine if *S. stercoralis* extract can elicit eosinophil chemotaxis, and to then compare the migration response, including second messenger signals and receptors, to those mechanisms triggered by host chemoattractants. **(2)** Eosinophils from mice deficient in the granule products MBP or EPO were tested for their ability to kill the parasite. **(3)** Parasite antigen-pulsed eosinophils were injected intraperitoneally into naïve or immunized mice, then examined for antigen specific immune responses.

Results: (1) Eosinophils exhibited chemotaxis to proteins and chitin derived from *S. stercoralis* extract. Pretreatment of eosinophils with pertussis toxin, an inhibitor of Gαi-family coupled signaling, inhibited migration of the eosinophils to the parasite extract. Blocking PI3K, Tyrosine kinase, p38 and p44/42 also inhibited eosinophil chemotaxis to parasite extract. Pretreatment of eosinophils with either a CCR3, CXCR4 or CXCR2 antagonist significantly inhibited eosinophil chemotaxis to the parasite extract. **(2)** Eosinophils deficient in MBP had decreased ability to kill the parasite in vivo and in vitro whereas eosinophils deficient in EPO killed the worms as effectively as wild type cells. **(3)** A single inoculation of antigen-pulsed eosinophils was sufficient to prime naïve mice and boost immunized mice for antigen-specific Th2 immune responses with increased IL-4 and IL-5 production. Mice inoculated with live eosinophils pulsed with antigen developed significant increases in parasite antigen specific IgM and IgG levels in their serum. Antigen-pulsed eosinophils deficient in MHC class II molecules or antigen-pulsed dead eosinophils failed to induce immune responses thereby demonstrating the requirement for direct interaction between eosinophils and T cells.

Conclusions: Eosinophils independently migrate to the parasite *S. stercoralis* utilizing intracellular second messenger signals and multiple receptors on the eosinophil surface. After entering the parasite microenvironment, eosinophils kill the larvae in the innate immune response through a MBP dependent mechanism. Finally, eosinophils pulsed with soluble parasite antigen can function as APC for the induction of the primary and the expansion of the secondary Th2 immune responses to *S. stercoralis* in mice.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – EOSINOPHILS AND DISEASE: PATIENTS TO ANIMAL MODELS AND BACK TO PATIENTS – ANIMAL MODELS

James J. Lee

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The pulmonary eosinophilia accompanying the pathologies of asthma has been a correlative feature recognized even in the earliest studies investigating this disease. Innumerable investigations have confirmed and detailed this relationship, demonstrating that the presence of eosinophils is predictive of disease severity and occurs even in mild cases. The recruitment of eosinophils also occurs in animal models of allergen-mediated respiratory inflammation; the mouse, in particular, has been extensively studied. Despite the availability of mouse models that correlate pulmonary eosinophilia with histopathology and lung dysfunction, and in some cases provide evidence of a causative relationship, eosinophil effector functions are poorly understood. Indeed, questions remain as to the role(s), if any, of these leukocytes.

The goal of this ‘State-of-the-Art” review is to summarize the literature and highlight relevant observations linking eosinophils and allergic asthma using mouse model systems. We believe that although this relationship is complex, the plurality of data clearly supports hypotheses suggesting that eosinophil activities contribute to the pathological changes and immune responses associated with asthma.

INVITED SPEAKER ABSTRACTS

STEROID TREATMENT IN MOUSE MODELS OF CHRONIC EXPOSURE TO ALLERGEN

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Mouse models of asthma typically involve brief or chronic exposure to allergen, in order to further our understanding of specific aspects of the human condition. We have utilized models of chronic exposure to gain insight into the mechanisms underlying airway hyperresponsiveness [AHR].

Transient versus AHR: Following a period of chronic exposure to allergen, mouse display marked AHR compared to sensitized only mice. A component of this AHR will reverse with time, in parallel with the resolution of eosinophilic inflammation. This *transient* component of AHR can also be reversed by treatment with corticosteroids in the face of ongoing exposure to allergen. A further component of AHR persists after the cessat4ion of allergen exposure, and is associated with persistent structural changes in the airway, including increased smooth muscle and sub-epithelial fibrosis. This sustained AHR and the associated remodeling cannot be reversed by corticosteroid treatment.

Preventative Treatment with Corticosteroids: Treatment of mice with corticosteroids either topically or systemically is highly effective at preventing allergen induced eosinophilic inflammation. Provided this treatment is initiated at the onset of allergen exposure, and maintained throughout, it is highly effective at preventing the development of airway remodeling and the associated sustained component of AHR. This finding suggests potential disease modifying role for these drugs, not yet evident in clinical practice.

As discussed above concomitant treatment with corticosteroids throughout a period of chronic exposure to allergen can completely prevent the development of asthma related pathophysiologic changes. However, simultaneous cessation of allergen exposure and corticosteroid treatment results in a rebound increase in goblet cell numbers. This increase is completely IL-13 dependent, associated with development of AHR, but not associated with other inflammatory events, including eosinophilia. Whether this rebound phenomena is clinically relavent remains to be determined.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND DISEASE: PATIENTS TO ANIMAL MODELS AND BACK TO PATIENTS

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Background: Eosinophils have long been associated with asthma, but whether they contribute to or help control different aspects of disease remain uncertain. A variety of animal models have been developed to address different aspects of airway disease including airway hyper-responsiveness and airway remodeling.

Objectives: To relate differences in contributions of eosinophils to AHR and remodeling in different mouse strains to potential diversity of patients with asthma, and to document time course of eosinophil infiltration and activation of remodeling following allergen challenge.

Methods: Bronchial biopsies were obtained at baseline, then at 24hrs and later 7 days after inhaled allergen challenge of mild asthmatic volunteers. Immunohistochemistry, ISH and confocal microscopy were used to evaluate airway inflammation, remodeling, and TGFβ signalling. Results will be related to data from mouse studies.

Results: Eosinophil infiltration after allergen challenge peaked at 24 hours and had returned to baseline by 7 days after allergen challenge whereas AHR and activation of remodeling persisted.

Conclusions: The time course is important in studying associations of eosinophils with disease. Eosinophils, mouse strains and patients are all diverse and understanding genetic and other influences tending to eosinophilic and non-eosinophilic asthma as well as phenotypic differences between airway eosinophils in asthma, eosinophilic bronchitis and rhinitis will be important to future targeting of anti-eosinophil treatment.

This project was funded by a grant the Imperial Trust and GlaxoSmithKline.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION: GASTROINTESTINAL DISEASES – ANIMAL MODELS

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Eosinophilic esophagitis (EE) is an emerging worldwide disease that mimics gastroesophageal reflux disease (GERD) and can lead to esophageal narrowing and stricture, as well as tissue remodeling including fibrosis. EE is differentiated from GERD by the lack of response to acid suppression, the magnitude of mucosal eosinophilia and epithelial thickening, its male predominance and a high rate of association with atopy. A series of experiments have demonstrated that EE is an allergen (primarily food antigen)-driven Th2-cell dependent disease associated with abnormal keratinocyte production of eotaxin-3 and an associated EE transcriptome involving 1% of the genome. Recent studies in man and mice have defined the upstream pathway responsible in large part for the induction of EE-associated pathology, and have defined molecular and cellular targets for drug intervention. Early clinical trials have revealed that the EE transcriptome and pathology are largely reversible with therapeutic intervention.

INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND EOSINOPHIL CHEMOKINES, CCL11 AND CCL24 IN DSS-INDUCED COLONIC INJURY

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Background: Inflammatory bowel disease (IBD), Crohns disease (CD) and ulcerative colitis (UC) are chronic, relapsing, remitting gastrointestinal (GI) diseases. Elevated levels of eosinophils and eosinophil-derived granule proteins in colonic biopsy samples from UC patients have been shown to correlate with morphological changes to the GI tract, disease severity and GI dysfunction. Despite the evidence suggesting that eosinophils have a pathogenic role in IBD, the molecular processes involved in eosinophil recruitment into the colon and the biological significance of eosinophils in IBD are poorly understood.

Objectives: To examine the expression and role of the eosinophil chemotactic factors CCL11 (eotaxin-1), CCL24 (eotaxin-2) in eosinophil recruitment in an experimental dextran sodium sulfate (DSS)-induced colonic injury.

Methods: We subjected CCL11, CCL24 and eosinophil deficient mice to the established experimental model of colonic injury (2.5% DSS). Kinetic analysis of CCL11 and CCL24 mRNA expression in the colon was quantitated by northern blot analysis. The cellular source of CCL11 was determined by *in situ hybridization*, flow cytometry and immunofluorescence analysis. Eosinophil levels were quantitated by immunohistochemistry employing the anti-MBP antibody.

Results: DSS induced CCL11 (~10-fold) and CCL24 (~ 2-fold) mRNA expression in the colon of WT mice. The upregulation of CCL11 and CCL24 expression correlated with an increase in mucosal and sub-mucosal eosinophil levels over the 8 day experimental regime. DSS-induced colonic eosinophilic inflammation was abrogated in CCL11- and not CCL24-deficient mice demonstrating that CCL11 critically regulates eosinophil recruitment into the colon. Flow cytometry and *in situ hybridization* analysis revealed that DSS-induced CCL11 mRNA expression was primarily derived from $CD11b^+$ $F4/80^+$ cells. Furthermore, using eosinophil-deficient mice we demonstrate that eosinophils play a role in the induction of the pathophysiological features of colonic injury (colonic epithelial ulceration).

Conclusions: Eosinophilic inflammation associated with experimental colonic injury is predominantly regulated by $CD11b^+$ $F4/80^+$ intestinal cell derived CCL11. Furthermore, eosinophils play an integral role in the pathogenesis of disease injury. Collectively, these data implicate CCL11-mediated eosinophilia in experimental colonic injury and highlight the need for assessment of the role of eosinophils and eosinophil chemotactic factors in IBD.

This project was funded by a grant from Crohn's and Colitis Foundation Career Development Award and CCHMC Trustee /DDRDC Pilot and Feasability grant-Digestive Disease Research Development Center for Growth and Development (NIH R24 DK64403).

INVITED SPEAKER ABSTRACTS

MOUSE EOSINOPHILS ARE FUNDAMENTALLY DIFFERENT THAN HUMAN EOSINOPHILS

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From studies of allergic and inflamed airways in vivo it has emerged that mouse and human eosinophils may not differ much by the circumstances under which they are recruited to the airways. Nor may they differ in their intriguing modes of leaving the airway tissues (apoptotic eosinophils here are equally rare in either species). Airway eosinophilia is in fact a useful measure in mouse models and the human disease. Morrow Brown originally demonstrated efficacy of inhaled glucocorticoids in asthma. In a letter he explained that careful selection of asthmatics with sputum eosinophilia was crucial to his unprecedented success in those early days. However, actual roles of eosinophils concern their activity rather than their number. It is further postulated that activity in airway tissues is more important than that in the airway lumen. One compelling sign of accomplished activity is the appearance of degranulated eosinophil phenotypes. As defined by ultrastructural cell features, degranulation of eosinophils in human diseased tissues commonly occurs through piecemeal degranulation (PMD) and/or primary cytolysis (PCL). PCL correlates well with airway symptoms of human seasonal allergic disease, but is also associated with high speed epithelial restitution processes in vivo in guinea-pigs. By contrast, even massive exposure to allergen cannot induce PMD or PCL in allergic mice. Indeed, you have to use extreme means, such as stimulating the death (FAS-) receptors of those eosinophils that have accumulated in mouse allergic lung tissues, to see any degree of ‘degranulation’ and then the picture is that of secondary necrosis more than PCL or PMD. Lack of PMD and PCL conspicuously and fundamentally limits the use of mice in studies of roles of the eosinophil in the human disease.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION: EOSINOPHILS AND ASTHMA – PATIENTS

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The role and contribution of eosinophils to clinical asthma has, and continues, to undergo re-evaluation. There are a number of aspects of eosinophils, and their presence, that suggest they have a direct role in the pathophysiology of asthma. Both peripheral blood and sputum eosinophils show relationships to markers of asthma severity. Furthermore, with treatment of asthma and development of symptomatic control, peripheral blood eosinophils are reduced. This relationship is most commonly seen with corticosteroids but has also been noted with other treatments including leukotriene receptor antagonists, omalizumab, and other experimental therapeutics. Finally, sputum eosinophils increase in advance of an asthma exacerbation suggesting a “cause and effect” relationship. Conversely, directing treatment towards a reduction in sputum eosinophils is associated with lessened asthma exacerbations.

The biological role that eosinophils assume in asthma is less well defined. Models, such as antigen challenge, find associations between the appearance of airway eosinophils to the development of the late pulmonary response and enhanced airway hyperresponsiveness. However, reducing circulating and sputum eosinophils does not always affect the development of the late phase response.

More recently, evidence has begun to emerge that eosinophils may function as immunomodulating cells and interact with other airway cells, such as lymphocytes, to direct the function of other cytokine/chemokine secreting cells. Finally, there is evidence that the eosinophil may be more important in the development of the airway tissue repair processes, i.e. interaction with fibroblasts, to promote collagen deposition. Thus, although often present in abundance in clinical asthma, the putative role of the eosinophil in this disease is still undergoing evaluation. To fully understand this cell’s role and contribution many aspects of asthma will need to be considered: location, state of activation, phenotype of the patient, and acute vs. persistent phase of the disease.

INVITED SPEAKER ABSTRACTS

HEMOPOIETIC STEM CELLS AS EARLY DETERMINANTS OF HEALTH AND DISEASE: RELEVANCE TO ATOPY AND EOSINOPHILIC INFLAMMATION

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Background: Rhinosinusitis and asthma involve systemic processes which include the active recruitment and differentiation of hemopoietic and non-hemopoietic progenitors (stem cells, SC), which 'sense' tissue injury and contribute to inflammation, repair and remodeling. Circulating SC can differentiate into inflammatory effector cells (such as neutrophils, eosinophils, basophils, mast cells and monocytes), or into non-structural and functional tissue cells, determined by locally elaborated growth factors in a process termed "*in-situ* hemopoiesis".

Objectives: To establish the role of eosinophil lineage-specific SC in the development and maintenance of the allergic inflammatory diathesis.

Methods: Methylcellulose colony-forming assays, multi-parametric flow cytometry and mRNA analyses by real-time polymerase chain reaction (Q-PCR) were employed.

Results: Allergen-induced kinetic changes can be documented in circulating and marrow populations of eosinophil-basophil (Eo/B) progenitors, including upregulation of IL-5Rα, CCR3 (eotaxin receptor) and CXCR4 (SDF-1 receptor) on bone marrow (BM) as well as airways tissue $CD34^+$ cells. SDF-1 plays a critical role in adult hemopoietic SC homing as it does during embryogenesis, highlighting that SC can respond to allergic stimuli, causing symptoms of allergic disease, and providing targets of therapy for these conditions. Moreover, activation of selective, eosinophil lineage-specific functional and phenotypic (e.g., in expression of IL-5Rα/GM-CSFRα on $CD34^+$ cells) alterations in cord blood (CB) SC has been observed at birth in neonates at risk for atopy and asthma, as well as in infants who subsequently develop fever and wheeze in response to acute respiratory infections. CB SC may thus provide *innate* immune response pathways in early life to allergenic and infectious stimuli. Recently we have shown that CB SC populations are altered in response to maternal skin prick test responses to common allergens and to dietary intervention during pregnancy, correlating with improved allergic outcomes in early life.This area promises to be of great interest in understanding the role and fate of the very abundant $CD34^+$ SC populations present in CB at birth. Q-PCR has revealed kinetic patterns of expression of Eo/B-lineage specific genes, specifically: GATA-1, MBP and IL-5Rα. Stimulation with IL-5 results in an early up-regulation of GATA-1 expression, peaking at 24-48h. In contrast, MBP is up-regulated in a slowly progressive pattern, maximally at 72h, while there is stable, low expression of IL-5Rα (with increase over time of the soluble, and decrease of the transmembrane, isoforms).

Conclusions: These results reveal novel mechanisms for predicting the generation of tissue airway eosinophilic inflammation in infancy and early childhood, in response to viral or allergenic stimuli. Molecular markers of critical differentiation-specific events in CB SC may herald future atopic and asthmatic biological and clinical outcomes.

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INVITED SPEAKER ABSTRACTS

ROLE OF EOSINOPHILS IN ASTHMA

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Asthma is a condition which has three main characteristics, airflow obstruction which has a degree of variability, airway hyperresponsiveness (AHR) and airway inflammation which is usually eosinophilic. It is a heterogeneous condition which has at least five pathophysiological phenotypes. i) a 'classical asthma' pattern of variable airflow obstruction and airway hyperresponsiveness, ii) fixed airflow obstruction, iii) bronchitis, iv) severe exacerbations and v) bronchiectasis. Any patient can have one or more of these phenotypes and the likelihood of developing them depends in part on the severity of the disease. Each of these patterns of disease has a clinical, physiological and pathological correlate and each responds differently to treatment. The current evidence suggests that eosinophils do not have a major role to play in the classical asthma phenotype which is closely associated with mast cell infiltration of the airway smooth muscle (1,2). However eosinophils are closely associated both with severe exacerbations and the response to glucocorticoids (GC). The distinction between the 'asthma' phenotype and the 'severe exacerbation' phenotype was revealed by using cluster analysis to investigate phenotypic heterogeneity in asthma. Three groups identified using this objective, statistical method of analysis were a very symptomatic group without airway eosinophils (non-eosinophilic group), a group with symptoms and an airway eosinophilia (concordant group) and a group with an airway eosinophilia but few symptoms (inflammation predominant). Applying this analysis to a previous study of the value of controlling airway eosinophilia in preventing severe exacerbations (3) we found that most of the benefit was seen in patients who clustered to the inflammation predominant group. Consistent with this observation in a group of severe asthmatics the improvement in FEV_1 after a course of GC was closely associated with the baseline BAL eosinophil count. This data confirms the close association between eosinophilic inflammation and severe exacerbations but doesn't show a causal relationship. To determine if eosinophils are directly involved in causing the exacerbations we are currently undertaking a 12 month DBPC trial of anti-IL-5 in patients with inflammation predominant disease to determine if this drug will prevent severe exacerbations. How an airway eosinophilia might lead to severe exacerbations needs further investigation

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INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION: GRANULES IN THE GUT – THE IMPACT OF EOSINOPHILS ON THE GASTROINTESTINAL TRACT IN 2007

Glenn T. Furuta

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Over 50 years ago, the advent of fiberoptic endoscopy simplified the evaluation of the intestinal mucosa, allowing for the identification of eosinophilic inflammation. Since then, gastrointestinal eosinophilia has posed interesting and problematic questions for scientist and physician alike.

What is the significance of mucosal eosinophilia? Although eosinophils normally reside in the GI tract, pathologist now associate increased numbers of eosinophils with diseases including food allergies, inflammatory bowel disease, intestinal infections and eosinophilic gastrointestinal diseases (EGIDS). EGIDS are a unique group of diseases characterized by eosinophilic inflammation of the mucosa, muscularis or serosa that occur without an obvious etiology. Exact diagnostic criteria for EGIDs remain points for discussion.

How do eosinophils contribute to intestinal dysfunction? While specific answers are not yet available, one example of a potential structure-function relationship lies in the most common form of EGID, eosinophilic esophagitis (EE). Patients with EE present with gastroesophageal reflux like symptoms, dysphagia and food impaction and have dense esophageal eosinophilia. Endoscopic, manometric, ultrasonographic and radiological evaluations suggest that eosinophils impact esophageal motor function leading to either intermittent contractions or permanent tissue remodeling as a cause for symptoms.

One of the remaining dilemmas for clinician and scientists relates to optimal treatment. Presently, corticosteroids and nutritional management are the only effective treatments. Since no quality of life studies have been performed and the natural history of chronic intestinal eosinophilia is presently uncertain, treatment endpoints (symptom relief, histological remission) remain unresolved.

While much progress has been made in the identification and treatment of patients with EGIDS, future progress depends on collaborative efforts in multi-disciplinary, multi-centered studies.

INVITED SPEAKER ABSTRACTS

ALLERGIC EOSINOPHILIC GASTROENTERITIS WITH PROTEIN-LOSING ENTEROPATHY: CLINICAL FEATURES, INTESTINAL PATHOLOGY AND POSSIBLE MECHANISMS OF ALLERGY

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A subset of patients with allergic eosinophilic gastroenteritis (AEG) has anemia and hypoalbuminemia caused by protein-losing enteropathy (PLE). A retrospective medical record review of these patients followed by phone contact for clinical update revealed an excellent response to therapy with amino acid-based formula in all patients. Although they tolerated gradual introduction of some foods with time, food-responsive disease persisted in all patients over 2.5 to 5.5 years of follow-up. When histological features of intestinal and gastric biopsies of patients with AEG+PLE were compared to those with AEG but no PLE, significantly more mast cells were found in intestinal biopsies of the AEG+PLE group despite comparable number of eosinophils. In contrast, in gastric biopsies, eosinophils were more prominent in AEG+PLE, but mast cell numbers were similar in both groups. Blood and protein losses in patients with AEG+PLE are likely to be secondary to an increase in intestinal permeability; supported by a lactulose/mannitol/sucrose intestinal permeability test that demonstrated an increase in intestinal rather than gastric permeability. We hypothesize that intestinal mast cells cause the increase in intestinal permeability. The importance of mast cells in antigen-induced increase in intestinal permeability was previously demonstrated in a rat model of late-phase intestinal allergy. It is possible that transcytosis of antigen-IgE complexes by CD23a on human epithelial cells plays an important role in mast cell activation and increased intestinal permeability. Food-specific IgE is present in the intestinal lumen of patients with food allergy, as evidenced by elevated fecal levels of food-specific IgE following an oral food allergen challenge. CD23a was found to be expressed by primary human intestinal epithelial cells when assessed by RT-PCR. In polarized human T84 cells, CD23a was found to act as a bidirectional transporter of IgE. However, CD23-IgE complexes could traffic only in an apical to basal direction, bypassing lysosomal degradation in the epithelial cell. Antigen-IgE complexes delivered across the epithelial barrier were capable of inducing degranulation of rat basophil leukemia cells transfected with the human high-affinity IgE receptor. These results show that in the presence of IgE, CD23a that is expressed normally in human intestinal cells can function as an antigen-sampling mechanism capable of activating subepithelial mast cells. Mast cells in turn may induce increased intestinal permeability in patients with AEG+PLE.

INVITED SPEAKER ABSTRACTS

ANTI-IGE TREATMENT OF EOSINOPHIL ASSOCIATED GASTROINTESTINAL DISORDERS

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Background: Eosinophil Associated Gastrointestinal Disorders (EGIDs) are commonly associated with atopy and are being recognized with increasing frequency. Current therapy for EGIDs is inadequate.

Objective: We sought to determine the efficacy of anti-IgE therapy in EGIDs and investigate the role of IgE in disease pathogenesis.

Methods: Nine subjects with EGIDs received omalizumab every 2 weeks for 16 weeks while other therapy was held constant. Blood absolute eosinophil counts, tissue eosinophil counts, symptom scores, and free IgE were serially measured. Allergen skin testing, and flow cytometry for basophil activation and FcεRI were determined at baseline and at week 16.

Results: Omalizumab was associated with a decrease in absolute eosinophil count at both the 16 week (34%, p=0.004) and combined weeks 12-16 (42%, p=0.012) time points. Tissue eosinophils decreased in the duodenum (59%) and gastric antrum (69%), but did not reach statistical significance (p=0.074 and 0.098, respectively). Esophageal eosinophil counts remained unchanged. Basophil and dendritic cell FcεRI expression, and free IgE were all significantly decreased (p<0.005). Omalizumab increased the concentration of allergen required to trigger half-maximal basophil activation by 170-fold. Allergen skin test wheal and erythema responses decreased by 78% and 82%, respectively. Symptom scores were decreased at both the midstudy (63%) and end of study (70%) time points (p<0.005 for both).

Conclusion: These results demonstrate that IgE-mediated processes contribute to the generation of eosinophilic inflammation in EGIDs, and suggest that anti-IgE therapy may be effective in these disorders.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION: EOSINOPHILS AND CANCER – EOSINOPHILS AND MACROPHAGES REGULATE DAMAGE ASSOCIATED MOLECULAR PATTERN [DAMP] RESPONSE IN CANCER

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Background: Eosinophilic granulocytes, at increased numbers, are found within tumor tissues and the blood of cancer patients. Little is known, however, about their role in this setting and which factors promote their recruitment and trafficking to sites of tumor and necrosis. HMGB1 (high mobility group protein B1) is a member of the damage associated molecular pattern molecules (DAMPs) released by necrotic tumor cells but also actively secreted by activated monocytes.

Objectives/Methods: We demonstrate, in addition to TLR-4, eosinophils and macrophages also express another HMGB1 receptor, RAGE. We used human PBMC and isolated eosinophils to evaluate the response to tumor lysates and HMGB1. Human granulocytes were purified from whole blood by density gradient centrifugation using Ficoll-PaqueTM Plus (Amersham, Biosciences) followed by lysis of red cells using ammonium chloride solution (155 mM NH4Cl, 10mM KHCO3, and 0.1 mM EDTA). Human eosinophilic granulocytes were negatively selected out of the granulocyte population using MACS-separation (Miltenyi Biotec Inc.) following the manufacturer's instructions. The purity was assessed by H&E staining and was at least 95%. Cells were kept in RPMI 1640 (Cellgro; Mediatech Inc., Herndon, USA) supplemented with 10% FBS (Gibco-Invitrogen, USA) and containing 100U/ml Penicillin-G, 0.25µg/ml Amphotericin B, 100µg/ml Streptomycin (Cellgro; Mediatech Inc., Herndon, USA) in an humidified atmosphere at 37°C with 5% CO2. For experiments assessing peroxidase release phenol-red free IMDM (HyClone, Utah, USA) without serum was used. All media were supplemented with 10µg/ml polymyxin B (Sigma, USA) in order to block any effects of contaminating endotoxin.

Generation of necrotic colorectal tumor cells: Two individual colorectal tumor cell lines, HCT-116 and CaCO2 cells, were resuspended in PBS at a concentration of 1x107 cells/ml and lysed by 3 cycles of freeze-thawing (-80 to 37°C) followed by sonicating and repeated passage through a 28G needle. The viability following treatment was assessed using trypan blue exclusion and was always less then 0.1%. For colorimetric measurement of EPO release, lysates were spun down hard (16,300x g) and the soluble supernatant was used.

Results: Subsequently we demonstrated that both natural and recombinant HMGB1 serve as survival factors and chemoattractants for granulocytes and in particular for eosinophils. HMGB1 promoted eosinophil degranulation with release of eosinophil peroxidase and major basic protein. We show that peroxide abolishes the effect of necrotic tissue on eosinophils in terms of further release of peroxidase as well as major basic protein, a factor promoting opsonization of cellular debris. We describe a new role for eosinophils in "sensing necrosis" and facilitating oxidation and removal of cellular debris. Necrotic cells modulate immune responses. Similarly, incubation of peripheral blood mononuclear cells with either HMGB1 or tumor lysate induced expression of novel

miRNAs, which are rapidly upregulated. We have identified several putative miRs involved in HMGB1-induced signaling and/or differentiation including hsa-mir-155, hsa-mir-545 and let-7g. These miRs are especially interesting to us, because of their computationally calculated potential targets (which can be found on *http://www.microrna.org/mammalian/index.html*). Hsa-mir-155 has computationally predicted targets of Spi-1 (or PU.1) and TLR4, as well as MAP4K5 (a Mitogen-activated protein kinase) while hsa-545 has computationally predicted target of IRF-8. Both Spi-1 and IRF-8 are myeloid-specific transcription factors involved in monocyte activation and differentiation. The MAP Kinase may be involved in early HMGB1 signalling, which in turn may be regulated by miR-155. Interestingly, let-7g is one of the upregulated microRNAs. This microRNA has putative targets of IRF-5 (an intermediate in TLR signaling) and Spi-1/PU.1.

Conclusions: Our results suggest a plausible role for eosinophils and macrophages infiltrating tumor tissue. In addition, these findings have implications for the immunotherapy of patients with cancer and suggests a linkage between the evolution of tumor necrosis, the host response, and carcinogenesis.

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INVITED SPEAKER ABSTRACTS

EOSINOPHILS AND ALLERGIC INFLAMMATION: FROM ANGIOGENESIS TO HYPOXIA AND BACK

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Background: Allergic inflammation (AI) is characterized by the presence of chronic inflammation, remodelling and angiogenesis. Eosinophils, key players of A.I., have been shown to produce pro-angiogenic factors and to have pro-angiogenic properties in vitro. Nasal polyps (NP), a chronic condition of the upper airways present in chronic rhinosinusitis and common in asthma, are characterized by the presence of eosinophils that are the main inflammatory cell infiltrate, by angiogenesis and the expression of VEGF and its receptors. An important overlooked component of A.I. and the main stimulus to angiogenesis is tissue hypoxia, due to the lack of blood flow in the inflamed tissue which is caused by disruption of the blood vessels and the requirement of more blood to the proliferating tissue.

Objectives: The aim of the study was to assess the functional activity of eosinophils under hypoxic conditions in vitro and to correlate it to hypoxia and angiogenesis of NP.

Methods: Highly purified human peripheral blood eosinophils (MACS) were cultured with or without GM-CSF (20ng/ml) in normoxic (21% 0_2) or hypoxic (3% O_2) conditions and apoptosis (Annexin V/PI), production of cytokines (ELISA), MAPK phosphorylation (SDS-PAGE /WB), expression of HIF-1α (FACS/WB) were performed at various incubation times. Slides of NP surgical samples were stained with H&E, Kimura's and anti-HIF-α1 antibodies.

Results: Eosinophils incubated in hypoxia with or without GM-CSF were still viable (75.3± 7.0 % and 69.7 ± 13.4%) at 24 h, although at this time point 29.2 ± 0.9% of the cells were apoptotic vs. only 10.1 ± 7.5% in normoxia. Addition of GM-CSF decreased the hypoxia induced apoptosis to 14.9 ± 1.2% ($p<0.005$). Hypoxia increased significantly the release of both VEGF and IL-8 ($p < 0.005$) upon o.n. incubation, an effect significantly increased by the presence of GM-CSF in the medium. HIF-1α positive eosinophils in hypoxia were 52.16% vs 14.45% in normoxia (3 hrs). GM-CSF displayed a synergistic effect on the hypoxia mediated upregulation of HIF-1α. Hypoxia induced protein tyrosine phosphorylation (2 and 6 hrs) and the presence of GM-CSF upregulated ERK 1/2 and p38 phosphorylation (1 and 3 hrs). NP tissues were found to express high levels of HIF-1α that colocalized mostly with the eosinophils.

Conclusions: We have demonstrated that an hypoxic environment rich of GM-CSF enhances eosinophil functions in vitro. Under this condition eosinophils survive, produce VEGF and IL-8 and express HIF-1α. Also in NP and the hypoxic transcription factor HIF-1α is mostly associated with eosinophils. We therefore foresee a scenario in A.I. in which eosinophils are influenced by hypoxia in synergism with GM-CSF to enhance angiogenesis and possibly other eosinophil properties.

INVITED SPEAKER ABSTRACTS

EOSINOPHIL TISSUE INFILTRATION OF TUMORS IS A UBIQUITOUS PHENOMENON CHARACTERISTIC OF BOTH MOUSE TUMORS AND HUMAN CANCERS

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Rationale: Cancers are often associated with localized inflammatory responses at the sites of tumorigenesis. This localized inflammation has been hypothesized as both a host response to tumor growth and a nucleating site providing a microenvironment that promotes tumor onset and growth. Tumor associated inflammation is often accompanied by the influx and activation of pro-inflammatory leukocytes; macrophages are a particular well characterized and documented example. However, infiltration of tumors by eosinophils also appear to be common, and recent studies have suggested that this infiltration potentially modulates cancer growth rates as well as rate of metastasis.

Methods: We have developed novel anti-eosinophil polyclonal and monoclonal antibodies that have allowed us to systematically examine formalin-fixed paraffin-embedded sections from both cancer patients and mouse tumors. Moreover, through the use of mouse models modulating circulating eosinophils levels we have used established models of tumorigenesis to demonstrate potential links with tumor growth but not cancer metastasis.

Results: Immunohistochemistry using anti-eosinophil specific antibodies was used to examine tumor biopsies from cancer patients and tumors from mouse models. These systematic surveys have shown that virtually all human cancers are associated with a robust eosinophil infiltrate that is often accompanied by evidence of eosinophil degranulation. Interestingly, this ubiquitous eosinophil infiltrate also extends even to brain cancers despite the immune-privileged character of this area. Infiltration of eosinophils in tumors also was shown to be common among cancers in mice. These studies demonstrated that, similar to human cancers, eosinophil infiltrates of mouse tumors are spatially restricted and limited to the capsule regions surrounding tumors and, in particular, the necrotic areas of tumors. Additional studies in immundeficient *nude* mice show that this tumor associated eosinophilia occurs despite the absence of acquired immune responses, suggesting that the infiltrate is an inflammatory and not an immune response to the tumor. Subsequent studies using mice have shown that this tumor-associated eosinophilia was capable of modulating tumor-growth kinetics but is unlikely to have a role in metastasis of tumors to secondary sites.

Conclusions: Eosinophil infiltration of tumors is a ubiquitous phenomenon occurring in virtually all types of human cancers and mouse models of tumorigeneis. This infiltrate is an inflammatory response that is independent of T cell activities. Studies in mice also demonstrate that eosinophil activities may modulate tumor growth but have little to no role in the establishment of secondary sites associated with tumor metastasis.

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INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – MODELLING THE ROLE OF EOSINOPHILS IN THE REGULATION OF IMMUNE RESPONSES AND INFLAMMATORY DISEASES

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The eosinophilic leukocyte has been implicated in the regulation of fundamental immune processes, host defence against parasitic infestation and in the development of lesions associated with inflammatory diseases. To obtain further insights into the potential contribution of eosinophils to immune and disease processes animal models have been extensively employed. In recent years the development of transgenic and factor deficient mice has allowed the dissection of the contribution of eosinophils and their regulatory molecules in a range of disease models and in the regulation of immune processes. However outcomes of such studies have at times produced conflicting and controversial data that has failed to unequivocally resolve questions on the functional contributions of eosinophils. In part these conflicting outcomes can be explained by detailed analysis of what an individual model represents in terms of pathogenic mechanisms and in the temporal analysis of responses. In this presentation an overview of studies that focuss on the role of eosinophils in inflammatory and immune processes will be presented with the aim of defining their potential relationship with inflammation, remodelling and pathophysiology

INVITED SPEAKER ABSTRACTS

ADENOSINE SIGNALING AND THE REGULATION OF PULMONARY INFLAMMATION; CONTRIBUTION OF THE A_3 ADENOSINE RECEPTOR AND EOSINOPHILS

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Background: Adenosine is a potent cellular signaling molecule that is generated in response to cellular stress and injury. Adenosine levels are elevated in the lungs of asthmatics where it is thought to exacerbate airway inflammation and remodeling. Extracellular adenosine regulates cell function by engaging cell surface adenosine receptors. Expression of the A_3 adenosine receptors (A_3R) is elevated in the airways of asthmatics and has been implicated in the regulation of inflammatory processes such as cellular migration and degranulation. It is our hypothesis that elevated adenosine levels serve to modulate inflammatory cell function in the diseased lung by engaging the A_3R on key cells such as mast cells, eosinophils and bronchial airway epithelium.

Objectives: To examine the expression and function of the A_3R in in vivo models of chronic lung disease that are associated with adenosine elevations.

Methods: Two models of lung disease were used to examine the expression and function of the A_3R in vivo. Mice deficient in the enzyme adenosine deaminase (ADA) develop adenosine-dependent airway inflammation and remodeling and have been useful in examining pulmonary features regulated by adenosine. In addition, adenosine levels have recently been shown to be elevated in the bleomycin model of pulmonary inflammation and fibrosis suggesting this model may be useful for examining the contribution of adenosine signaling to pulmonary fibrosis. A_3R expression was monitored in these models using real time rtPCR and in situ hybridization. The contribution of the A_3R was assessed using A_3R antagonists and A_3R-deficient mice.

Results: Expression of the A_3R was found to be elevated in RNA extracts from ADA-deficient mice and mice exposed to bleomycin. Expression was localized to mucus producing bronchial epithelium and eosinophils. Treatment of ADA-deficient mice with an A_3R antagonist or mating ADA-deficient mice onto an A_3R-deficient background resulted in decreased eosinophils in the airways, but an accumulation of eosinophils in lung tissue. This was also associated with decreased mucin production in the bronchial airways. Similar results were found in the bleomycin model. Interestingly, eosinophil peroxidase levels were substantially elevated in the lavage fluid of wild type mice but not A_3R-deficient mice suggesting this receptor may influence eosinophil degranulation.

Conclusions: These studies suggest that elevations in lung adenosine levels can influence eosinophil trafficking and degranulation in the lung. Additional studies are needed to clarify the cellular and molecular mechanisms involved.

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INVITED SPEAKER ABSTRACTS

POLLEN NADPH OXIDASES IN ALLERGIC INFLAMMATION

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Background: We have previously demonstrated an important role of pollen NADPH oxidases in induction of allergic airway inflammation. Several questions that were not answered in the initial publication include: 1) What is the role of these oxidases in induction of allergic inflammation in other surfaces? 2) Since pollens are large structures, how do pollen NADPH oxidases reach lower airways to initiate allergic inflammation? 3) Do anti-oxidants overcome the potentiating effects of pollen NADPH oxidases in allergic airway inflammation? 4) What signaling proteins are induced by these oxidases?

Objectives: We tested the role of these oxidases in murine allergic conjunctivitis. We examined the role of subpollen particles and anti-oxidants in allergic airway inflammation.

Methods: To examine the role of pollen NADPH oxidases, BALB/c mice were sensitized and challenged with ragweed extract (RWE). Conjunctivitis was induced by challenge with RWE or whole pollen. Distilled water was used to induce generation of subpollen particles from ragweed pollen. Anti-oxidants were administered locally into the lungs of sensitized mice mixed with RWE challenge. Dissected lungs were homogenized and lysates were analyzed using a microarray of 500 antibodies. Proteins in PBS-challenged or RWE-challenged lysates were labeled with either Cy-3 or Cy-5.

Results: Pollen NADPH oxidases initiated and boosted inflammation in allergic conjunctivitis. Subpollen particles contain pollen NADPH oxidases and Amb a 1. Anti-oxidants inhibit ROS generation by pollen NADPH oxidases and induction of inflammation by these oxidases. RWE challenge induced AP endonuclease 1, c-fos, Heme oxygenase 1, p67-phox, and MAP kinases p38-delta and ERK-1.

Conclusions: Pollen NADPH oxidases play an important role in initiating and boosting of allergic inflammation in different mucosal surfaces. These effects can be reversed by local administration of anti-oxidants.

This project was funded by grants from NHLBI (RO1 and NO1) and NIAID (PPG).

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – ROLES OF EOSINOPHILS AND MECHANISMS IN TISSUE REMODELING AND FIBROSIS

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Eosinophils are now thought to play a significant role in mediating tissue remodeling and fibrosis in a variety of eosinophil-associated diseases including asthma, eosinophil myalgia syndrome, eosinophilic endomyocardial fibrosis, idiopathic pulmonary fibrosis, scleroderma, and eosinophilic esophagitis. Eosinophils are implicated in fibrogenesis through their clinical disease associations, their elaboration of fibrogenic growth factors such as TGF-β, IL-1β, PDGF-BB, and secretion of their granule cationic proteins including MBP, EPO, ECP, and matrix metalloproteinases, e.g. MMP-9. Eosinophils have been identified as the major TGF-β producing cell in the lung in human asthmatics and murine asthma models. Both human and animal model studies provide compelling evidence for eosinophils as effectors of tissue remodeling and fibrosis (Fig. 1). A reduction in bronchial mucosal eosinophils induced by treatment of asthmatics with anti-IL-5 antibody significantly decreased the expression of a number of ECM proteins including tenascin, lumican and type III collagen in the bronchial reticular basement membrane. Anti-IL-5 treatment also decreased tissue eosinophils and deposition of ECM proteins in the skin in allergen-induced late-phase reactions in atopics. Co-culture of eosinophils and fibroblasts induces fibroblast to myofibroblast trans-differentiation through secretion of TGF-β. Direct evidence for the role of the eosinophil in tissue remodeling and fibrosis comes from studies in which IL-5 knockout or eosinophil-deficient mice were used to demonstrate essential roles for eosinophils in the development of airway hyperreactivity (AHR) and airway remodeling including mucus (goblet) cell metaplasia, smooth muscle hyperplasia, and subepithelial fibrosis. Multiple cytokines expressed by eosinophils are implicated in the development of remodeling and fibrosis (Fig. 1). TGF-β is considered the most potently fibrogenic, and its expression is correlated with bronchial airway fibrosis and severity of asthma. Induction of pulmonary fibrosis by TGF-β, IL-1β, and others provides some of the best evidence for involvement of these factors in inducing remodeling and fibrosis in the lung and other tissues. Recent studies and novel insights into these fibrogenic mediators and the mechanisms by which eosinophils participate in the pathogenesis of remodeling and fibrosis will be reviewed.

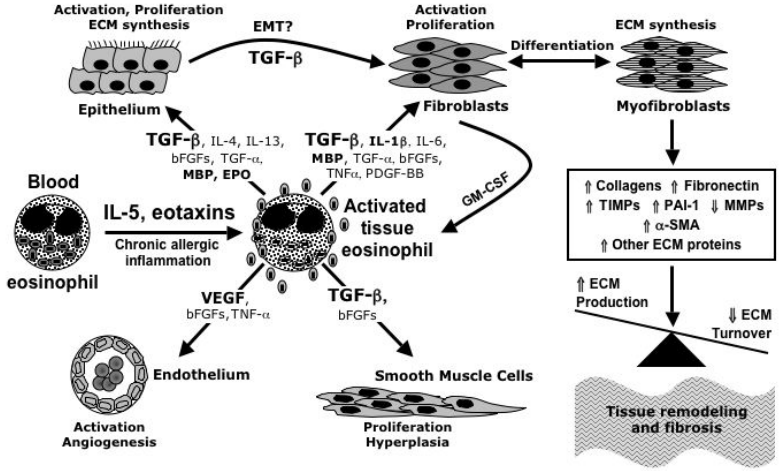

Fig. 1 – A paradigm for eosinophil participation in tissue remodeling and fibrosis.

Funded in part by NIH grants AI025230 and AI033043 to SJA.

INVITED SPEAKER ABSTRACTS

EOSINPHILS AND AIRWAY REMODELING: TGF-β-MEDIATED LUNG MYOFIBROBLAST DIFFERENTIATION AND BRONCHIAL EPITHELIAL-MESENCHYMAL TRANSITION

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Background: Eosinophils have been implicated in the airway fibrosis and epithelial alterations that occur in asthma, but the mediator(s) and signaling pathways involved have not been established.

Objectives: To determine the ability of human eosinophils to stimulate lung fibroblast-to-myofibroblast differentiation and epithelial-mesenchymal transition (EMT) in bronchial epithelial cells, and to determine the role of TGF-β and Smad signaling in these processes.

Methods: IMR-90 human lung fibroblasts and differentiated normal human bronchial epithelial (NHBE) cells (grown at air-liquid interface) were co-cultured with human peripheral blood eosinophils for up to 72 h. Real-time PCR, immunoblotting, and confocal immunofluorescence microscopy were used to determine expression of α-smooth muscle actin (α-SMA) in IMR-90 cells, and E-cadherin, β-catenin, vimentin, α-SMA, and Snail in NHBE cells.

Results: Co-culture with eosinophils stimulated increased expression of α-SMA in IMR-90 cells, indicative of differentiation to a myofibroblast phenotype. Eosinophil-induced myofibroblast differentiation was accompanied by activation of Smad3, and was blocked by an antibody to TGF-β_1 and an inhibitor of the TGF-β type I receptor (TβRI) kinase, but not by cysLT1 or cysLT2 leukotriene receptor antagonists. Co-culture of eosinophils with NHBE cells caused the epithelial cells to enlarge and become irregular in shape. This was accompanied by loss from cell-cell borders of the epithelial junction proteins, E-cadherin and β-catenin, and induction of markers of mesenchymal differentiation, including vimentin, α-SMA, and the transcription factor Snail, phenotypic changes indicative of EMT. Similarly, TGF-β_1 induced changes consistent with EMT in NHBE cells. Eosinophil-induced EMT was blocked by the TβRI kinase inhibitor.

Conclusions: Human eosinophils stimulate lung fibroblast-to-myofibroblast differentiation, and EMT in bronchial epithelial cells. Both processes are mediated by TGF-β released by eosinophils, and require TβRI-dependent Smad signaling in fibroblasts and bronchial epithelial cells, respectively. These data elucidate TGF-β-dependent mechanisms by which eosinophils may contribute to airway remodeling in asthma.

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INVITED SPEAKER ABSTRACTS

TISSUE REMODELING IN PEDIATRIC EOSINOPHILIC ESOPHAGITIS: WHO HAS IT AND DOES THERAPY HELP?

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Background: Eosinophils are associated with tissue remodeling in asthma. We recently showed that children with eosinophilic esophagitis (EE) and stricture formation also have features of tissue remodeling. Studies have not yet determined if children with milder EE have features of esophageal remodeling or if these features are modified by esophageal corticosteroid therapy.

Objectives: We utilized quantitative immunohistochemical analysis to determine if children with EE but without strictures have features of esophageal remodeling and to determine if these features are modified by esophageal budesonide therapy.

Methods: Endoscopically obtained esophageal biopsy specimens from pediatric patients meeting histologic criteria for EE who did not have stricture formation were evaluated for levels of eosinophilic inflammation, fibrosis, and vascular activation at baseline and following swallowed budesonide therapy.

Results: Children with EE but without stricture formation had features of esophageal remodeling including increased numbers of TGFβ_1 and phosphorylated-Smad2/3 positive cells, increased vascularity, and endothelial activation. However, the severity of remodeling features was less than that observed in strictured EE patients. Following budesonide therapy, certain features of tissue remodeling such as the number of TGFβ_1 positive cells were improved.

Conclusions: Children with non-strictured EE have features of tissue remodeling that can be modified with topical budesonide treatment. The severity of features of remodeling in non-strictured patients is milder than that seen in pediatric EE patients with stricture formation.

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INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – EOSINOPHILS AND DISEASES RELATED TO VIRUSES, FUNGI, AND OTHER PATHOGENS

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Background: Blood and tissue eosinophilia is a prominent and universally acknowledged feature of parasitic helminth infection. In contrast, the interactions of eosinophils with other pathogens, including viruses, bacteria, fungi, are not as familiar to most researchers, and are substantially less well-characterized.

Findings: Eosinophils are recruited to the lungs in response to infection with respiratory syncytial virus, and may be responsible for pathophysiologic responses to this infection, including acute wheezing and prolonged post-recovery asthmatic sequelae. At the same time, other studies suggest that eosinphils may be involved in host defense by virus clearance. Similarly, eosinophils and eosinophil cell lines can be infected by human immunodeficiency virus (HIV), and, although they are not major participants in the ultimate sequelae of HIV-disease, eosinophils are a prominent feature of the pruritic papular eruption (PPE) skin rash frequently associated with HIV infection.

Eosinophil-mediated responses to fungal infection, fungal colonization, and fungal-specific antigens have been explored both clinically and in mouse models. Both allergic bronchopulmonary aspergillosis (ABPA, involving allergic responses to *Aspergillus* mold sps.) and chronic fungal rhinositis (CRS, associated with allergic responses to *Alternaria*) are conditions in which hypersensitivity to fungal antigens results in severe symptomatic distress, in conjunction with prominent IL-5 mediated eosinophilia in respiratory tissues and secretions. Specific antifungal and systemic anti-inflammatory therapies show promise for the treatment of these conditions. In contrast, chronic fungal lung infection has been modeled in susceptible mouse strains via direct, intratracheal instillation of *Cryptococcus neoformans*. *C. neoformans* fungal pneumonia is associated with profound pulmonary eosinophilia and destruction of lung tissue, but evidence suggests that eosinophils are not promoting clearance of fungal components.

Finally, although there are a number of studies reporting the association of eosinophils and eosinophil secretory proteins with bacteria and bacterial infection, there is no convincing evidence supporting a role for eosinophils in innate immunity against bacterial infection.

Conclusions: The role of eosinophils in the pathophysiologies directly and indirectly related to virus infection and hypersensitivity to fungal antigens are intriguing and relatively unexplored areas for future research.

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INVITED SPEAKER ABSTRACTS

INNATE AND ACQUIRED IMMUNE RESPONSES TO ENVIRONMENTAL FUNGI AND THEIR POTENTIAL ROLES IN HUMAN CHRONIC AIRWAY DISEASES

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Background: The etiology of asthma and other allergic diseases is complex and multifactorial; it likely involves the interactions between genetic factors and environmental stimuli. Aeroallergens, such as mite, cockroach and fungi, smoking behavior, indoor and outdoor air pollution, and viruses are particularly important in the etiology and pathogenesis of asthma. Among various aeroallergens, an association between fungal exposure and asthma has long been recognized clinically since 1698. In particular, much evidence suggests an association between *Alternaria* and the development and exacerbation of asthma.

Objectives: We wanted to understand better the molecular mechanisms involved in immune responses to environmental fungi and their implications for human diseases.

Methods: The effects of *Alternaria* extract and germinating *Alternaria* spores on eosinophils and airway inflammation were studied by a series of in vitro experiments and animal models. The exposure and immune responses to fungal antigens were evaluated in the airways and in blood specimens obtained from patients with chronic rhinosinusitis (CRS), which is an airway eosinophilic condition closely related to asthma.

Results: We found that *Alternaria* extract potently stimulates activation and degranulation of human eosinophils. The degranulation was induced by aspartate protease-like activity contained in the extract and mediated by a protease-activated receptor (PAR), PAR-2, expressed on human eosinophils. Consequently, human eosinophils inhibited fungal growth and killed the fungus. When the *Alternaria* extract was administered intranasally to non-sensitized mice, it also caused marked airway eosinophilia, mucus production, IgE and IgA production, and airway hyperreactivity. Experiments with genetically deficient mice showed that the marked airway eosinophilia develops by the concerted action of both the innate immune response (i.e. NK cells) and the acquired immune response (i.e. CD4+ T cells). The protease activity in the extract was also implicated in the pathological changes of the mouse airways. Furthermore, patients with CRS showed increased cellular and humoral immune responses to environmental fungi, in particular *Alternaria* and *Penicillium*, as evidenced by increased production of IL-5 and specific IgG antibodies.

Conclusions: Induction of airway eosinophilia and activation of recruited eosinophils may be a part of the natural immune responses to environmental fungi and their secreted product(s). Dysregulated innate and/or acquired immune responses to these environmental fungi may be implicated in the development and exacerbation of chronic eosinophilic airway inflammation in patients.

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INVITED SPEAKER ABSTRACTS

EOSINOPHILS IN VIRUS INDUCED ASTHMA ATTACKS

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Eighty percent of asthma attacks in children and 50% of asthma attacks in adults are associated with viral infections. Eosinophilic inflammation and activation of eosinophils are commonly present in acute asthma, although eosinophilic inflammation is not a common response to most viral infections of the airways. Previous studies in mice suggested that sensitization to a non-viral antigen could cause CD8+ T-cells to produce IL-5 in response to a viral antigen, causing airway eosinophilia (Coyle et al, J Exp Med 181:1229, 1995). In a separate study (Graham et al, J Exp Med 180:1273, 1994), a TH2 response to influenza virus infection caused massive pulmonary eosinophilia in mice, associated with delayed viral clearance, increased lung injury, and markedly increased mortality.

Both viral infections and antigen inhalation cause airway hyperreactivity in experimental animals. In both cases, there is loss of function of inhibitory M2 muscarinic receptors on airway parasympathetic nerves, increasing acetylcholine release and potentiating reflex bronchoconstriction. In the case of antigen inhalation, this is caused by degranulation of eosinophils in contact with the airway nerves, releasing major basic protein, which binds to M2 receptors, blocking their function. In the case of viral infection, the cause of M2 receptor function is less clear, but is not eosinophil mediated. Rather, it is mediated by macrophages, and probably by release of interferons and TNF-alpha, down-regulating the expression of the M2 receptor gene.

We (Adamko et al, J Exp Med 190:1465-78, 1999) hypothesized that because many asthmatics are atopic, sensitization to non-viral antigens might lead to an eosinophilic response in those with virus induced asthma attacks. To test this, we sensitized guinea pigs to ovalbumin via intraperitoneal injection, but did not challenge them with inhaled ovalbumin. Instead, these sensitized guinea pigs, and unsensitized controls, were infected with parainfluenza virus. Both sensitized and non-sensitized animals developed airway hyperreactivity and M2 receptor dysfunction. However, in the case of the ovalbumin sensitized animals the M2 receptor dysfunction was mediated by release of major basic protein, and could be blocked by antibody to major basic protein, antibody to IL-5, or heparin (which binds major basic protein). Histological examination revealed that sensitization alone cause recruitment of eosinophils to the airway nerves, allowing them to be subsequently activated by viral infection. An unexpected finding was a substantial reduction in viral titers in the ovalbumin sensitized animals, which was reversed when eosinophils were depleted with antibody to IL-5.

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INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – ANTI-IL-5 THERAPEUTICS: RESULTS OF CLINICAL TRIALS IN ASTHMA

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Background: Interleukin (IL)-5 plays a pivotal role in eosinophilopoesis and survival. As a result, inhibition of IL-5 has been targeted as a possible therapeutic approach in asthma and other diseases in which eosinophils are thought to play an etiological role. Two different humanized monoclonal antibodies (hMoAb) have been developed and studied in humans.

Objectives: To evaluate the efficacy of anti-IL-5 hMoAb in asthma.

Methods: Review of available archival literature

Results: Initial studies with the hMoAb, mepolizumab used allergen inhalation challenge to evaluate its activity. The study demonstrated that treatment with the anti-IL-5 hMoAb significantly reduced the number of blood eosinophils and sputum eosinophils for at least 4 weeks. This was important information, as it confirmed a central role for IL-5 in the development of blood and airway eosinophilia following allergen inhalation, but the hMoAb did not have any significant effect on allergen-induced airway responses. However, other studies with mepolizumab demonstrated that treatment reduced the number of circulating and sputum eosinophils markedly, but the number of airway tissue eosinophils were reduced by only 55% and the number of bone marrow eosinophils by 52% over 20 weeks of treatment. Yet another study has demonstrated that treatment with mepolizumab reduced the levels of extracellular matrix proteins tenascin, lumican, and procollagen III in the bronchial mucosal reticular basement membrane in airway biopsy specimens of asthmatic subjects, and was associated with a significant reduction in the number of airway eosinophils expressing messenger RNA for TGF-ß and with decreases in the concentration of TGF-ß in BAL fluid, implying the existence of a role for eosinophil release of TGF-ß in airway remodeling in patients with allergic asthma.
Mepolizumab has also been evaluated in patients with persistent asthma in a study that, as yet, has been reported only in abstract form. The study included > 300 patients with poorly controlled asthma, and treatment with mepolizumab did not improve any indexes of asthma control; however, the higher dose of mepolizumab did significantly reduce the risk of the development of a severe asthma exacerbation by about 50%. A second anti-IL-5 hMoAb (SCH55700) has also been evaluated in a group of patients with severe asthma who were not responding to conventional asthma treatment, including high doses of inhaled or oral corticosteroids. The study demonstrated that the antibody reduced the number of circulating eosinophils and provided a small, but significant, improvement in FEV_1 after treatment with the lower dose, but no other clinical improvement was seen.

Conclusions: The patients studied in the anti-IL-5 hMoAb trials have been those with moderate-to-severe persistent asthma who are already using a large amount of antiasthma medication. Also, it is not obvious from these reports whether the patients had airway eosinophilia associated with poor asthma control. Studies are now underway evaluating the efficacy of mepolizumab in patients with difficult to manage asthma who have a persisting airway eosinophilia despite optimal conventional asthma treatment.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – ANTI-IL-5 THERAPEUTICS: CLINICAL TRIALS IN HYPEREOSINOPHILIC SYNDROMES

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Although eosinophilia is associated with a wide variety of conditions, including asthma and atopic disease, helminth infections, drug hypersensitivity, and neoplastic disorders, the role of the eosinophil in disease pathology is most clear in a heterogeneous group of disorders collectively referred to as hypereosinophilic syndromes (HES). Until recently, standard treatment of HES was limited to prednisone, hydroxyurea and interferon. With the development of anti-IL antibodies for treatment of asthma, however, the possibility of therapy targeted directly at eosinophils became available. Early case reports and small series of treatment of HES with these antibodies (mepolizumab and SCH557000) showed promising results. Consequently, a multicenter randomized, placebo-controlled trial of mepolizumab for the treatment of HES was undertaken. This trial confirmed the safety and efficacy of anti-IL5 therapy for the treatment of HES and provided the first example of successful therapy targeting eosinophils in an eosinophil-mediated disorder.

INVITED SPEAKER ABSTRACTS

STATE OF THE ART PRESENTATION – FORMULA FOR SUCCESS: CONNECTING WITH YOUR NIAID PROGRAM OFFICER

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Background: The National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports basic and applied research in an effort to better understand, treat, and ultimately prevent infectious, immunologic, and allergic diseases. The Division of Allergy, Immunology and Transplantation (DAIT) is responsible for national and international extramural research programs in basic immunology, and in the etiology, treatment, and prevention of immune-mediated diseases, including autoimmune diseases and asthma and allergic diseases.

Objectives: To provide an understanding of the people and resources at the NIH and NIAID that are available to help investigators secure NIH sponsored funding.

Methods: We will describe the roles of extramural personnel such as, Program Officers (PO), Scientific Review Administrators (SRA) and Grants Management Specialists (GMS). We will also review the many NIH sponsored funding opportunities such as RO1 and K awards.

Results: Investigator initiated research is shepherded through three separate entities; Center for Scientific Review (CSR), Program and Grants Management. The SRA provides scientific, administrative and logisitical oversight of the CSR peer review process. Program Officers serve as advocates for investigators and can provide guidance before and after submission of applications. Once funded, the PO becomes the primary scientific and service contact for the grant. All funding and administrative issues are managed by the GMS. Some of the available funding mechanisms include the NIH Director's New Innovator Award created this year that addresses two important goals: stimulating highly innovative research and supporting promising new investigators.

Conclusions: A better understanding of the funding mechanisms and the available extramural staff, at the NIH and NIAID, can help alleviate undue stress associated with the pursuit of research funding.

INVITED SPEAKER ABSTRACTS

TOWARDS REVERSIBLE TEMPORAL DELETION OF EOSINOPHILS *IN VIVO*

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Background: The eosinophil is a multifunctional cell that can be protective against parasite infestations [1], certain tumours [2], as well as viral infections [3]. However, the eosinophil is also implicated in the exacerbation of asthma (see [4,5]). Although a role for the eosinophil is indicated in all of these studies the precise mechanism of action in each circumstance is not known. Recently a transgenic mouse was generated that expresses a death gene (diptheria toxin, DTA) from an eosinophil specific promoter (eosinophil peroxidase, EPO) thereby completely deleting this cell type. Studies could therefore be performed in the total absence of eosinophils to help determine their role [5]. However, it would be of great benefit to be able to delete eosinophils, at will, at different times during development or adult life, and also to reinstate them after depletion. To this aim we are trialing a method [6] that is an adaptation of the Lac Operon system of *E coli* where gene transcription can be inhibited by the binding of a repressor protein (LacIR) to specific operator sites (LacO) in the promoter. This situation is reversible since in the presence of lactose (or its lactose analogue, IPTG) the inhibitor protein comes off the LacO sites and transcription can proceed. Using a modification of this system for mammals and whole body luciferase imaging, it has been demonstrated that, in mice transgenic for a Huntingtin LacO modified luciferase reporter construct (HDOSluc) and crossed with LacIR transgenic mice, gene function can be reversibly regulated in unborn pups *in utero* by the addition or removal of IPTG in the drinking water [7].

Objectives: To assess the efficacy of the LacO/LacIR system in the HDOSluc and LacIR mice. To generate mice transgenic for a LacO modified tissue specific promoter and assess the efficacy of the system. To modify this system to specifically control eosinophils.

Methods: We have compared luciferase activity in the HDOSluc mice in the presence of the LacIR repressor protein +/- IPTG in the drinking water. We have introduced LacO sites into a muscle specific promoter and measured reporter expression (EGFP) in transgenic mice in the presence of the LacIR repressor protein +/- IPTG in the drinking water. Moreover, we have introduced LacO sites into the EPO-DTA construct [5] (a kind gift from Jamie Lee) and are generating transgenic mice in order to specifically regulate the survival of eosinophils.

Results: In all tissues tested we see greater than 90% repression of luciferase activity in HDOSluc reporter mice when crossed with the LacIR repressor mice. Moreover, repression can be reversed (de-repressed) by the addition of IPTG in the drinking water by up to 80% within 48 hours in some tissues. Similarly, in the muscle specific LacO EGFP transgenic mice we see EGFP expression only in smooth muscle, and this can be repressed by LacIR and de-repressed by IPTG. LacO.Epo.DTA ES cells have been generated and injected.

Conclusion: These data indicate that tight reversible tissue specific control of gene expression is possible with the LacO/LacIR system by the simple addition or removal of IPTG in the drinking water. Reversible control of eosinophils may therefore be possible.

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INVITED SPEAKER ABSTRACTS

THIOCYANATE-DEPENDENT INHIBITION OF SPONTANEOUS AND AGONIST-INDUCED EOSINOPHIL APOPTOSIS AND DEGRANULATION BY EOSINOPHIL PEROXIDASE (EPO): A POTENTIAL PHYSIOLOGIC ROLE FOR ENDOGENOUSLY GENERATED HOSCN IN MAINTAINING EOSINOPHIL VIABILITY

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Background: Myeloperoxidase (MPO) mediates PMN apoptosis by generating HOCl, but the possible role of eosinophil peroxidase (EPO) in EO apoptosis is unexplored. *In vivo,* bromide (Br^-), nitrite (NO_2^-), and thiocyanate (SCN^-) compete for oxidation by EPO and H_2O_2 yielding, respectively, HOBr, NO_2°, and HOSCN. We have shown that SCN^- is the strongly preferred substrate for EPO *in vivo* and that HOSCN, in striking contrast to HOBr, NO_2° and HOCl, is a weak, sulfhydryl-specific oxidant. We recently showed that HOSCN is a uniquely potent phagocyte oxidant activator of endothelial cell NF-κB and transcriptional upregulation of tissue factor, E-selectin, ICAM-1, and VCAM-1. NF-κB activation typically inhibits apoptosis.

Objectives: To test the hypothesis that EPO, by generating HOSCN and activating NF-κB, antagonizes EO apoptosis and secondary necrotic degranulation.

Methods: Human blood EOs were incubated in RPMI medium and 10% FCS ± the various EPO substrates. Apoptosis was assayed by annexin V and propidium iodide staining with flow cytometry and confirmed by caspase 3 activation and morphology. EO degranulation was assessed by EDN ELISA. EO IκBα degradation and NF-κB p50 subunit nuclear translocation in nuclear extracts were analyzed by western blot.

Results: EOs cultured 2 days with 1 mM SCN^- were 69% viable, a 77% relative increase ($n=9$, $p < 0.0001$) over EOs cultured with nothing (i.e., Cl^- buffer control), 1 mM Br^-, or 1 mM NO_2^-, (all ~ 39% viable). When 0.5 nM PMA was added to activate the respiratory burst (a low threshhold dose), viability with SCN^- after 2 days was 63%, Cl^- 5%, Br^- 2%, and NO_2^- 14%. Viability with PMA and SCN^- was 20% higher than that with Cl^- without PMA ($p < 0.05$), suggesting that HOSCN not only fails to promote apoptosis but instead engenders an anti-apoptotic tone. Compatible with this, the EPO inhibitor azide (1 mM) abrogated the protective effect of SCN^- with or without PMA. EDN degranulation by PMA-activated EOS with SCN^- was 30-50% of that seen with the other EPO substrates. In comparison with Cl^-, unstimulated EOS incubated 60 min with SCN^- had pronounced IκBα degradation and Br^- was intermediate. EOs exposed 2h to 150 μM reagent HOSCN had substatially increased nuclear extract p50 levels above buffer control.

Conclusions: We conclude that HOSCN generated endogenously in EOs by the EPO/H_2O_2/SCN^- system plays a previously unsuspected role to maintain both constitutive and agonist-stimulated EO survival by a mechanism that may involve NF-□B activation. By blocking EO apoptosis, this system also inhibits potentially deleterious secondary necrotic degranulation. This substrate-specific function of EPO stands in stark contrast to the powerfully pro-apoptotic regulatory role played by MPO-derived HOCl in PMN.

CUTTING EDGE ORAL ABSTRACTS

TRANSDUCTION OF PTEN INTO EOSINOPHILS ATTENUATES SURVIVAL, CHEMOTAXIS, AND EOSINOPHILIC INFLAMMATION

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Background: Phosphatase and tensin homologue deleted on chromosome ten (PTEN) is part of a complex signaling system that affects a variety of important cell functions. PTEN blocks the action of PI3K by dephosphorylating the signaling lipid phosphatidylinositol 3,4,5-triphosphate. It has been shown that PTEN negatively regulates cell functions, such as proliferation, survival, and migration, in tumor and immune cells. Moreover, intratracheal administration of adenoviruses carrying PTEN cDNA inhibits OVA-induced eosinophilic inflammation and bronchial hyperresponsiveness in a murine model of asthma.

Objectives: We utilized TAT-fused protein transduction system to clarify the role of PTEN in eosinophils and allergic inflammation.

Methods: A small region of the HIV TAT protein (YGRKKRRQRRR), a protein transduction domain known to enter mammalian cells efficiently, was fused to the N-terminus of PTEN. Blood eosinophils were purified using Percoll and anti-CD16 antibody-coated magnetic beads. Eosinophil survival was analyzed by flow cytometry stained with Annexin V and propidium iodide. Chemotaxis assay was performed using Boyden chamber. BALF cell analysis and histological examination was performed using OVA-challenged A/J mice.

Results: We found that TAT-PTEN was successfully internalized into eosinophils and functioned as a phosphatase in situ. TAT-PTEN (1 μM), but not control protein TAT-GFP (1 μM), blocked the ability of IL-5 to prevent apoptosis of eosinophils from allergic subjects ($n = 3$, $p < 0.05$). Interestingly, the proapoptotic effect of TAT-PTEN was not observed in eosinophils from normal subjects. The eotaxin-induced eosinophil chemotaxis was inhibited by TAT-PTEN in a dose-dependent manner ($n = 4$). Intratracheal pretreatment of TAT-PTEN (3 μmol/mouse x 4 days), but not TAT-GFP, significantly inhibited the OVA-induced eosinophil infiltration in BALF ($n = 5$-6, $p < 0.05$). Histological examination of the lung including HE and alcian blue/PAS stainings revealed that TAT-PTEN, but not TAT-GFP, abrogated eosinophilic inflammation and mucus production.

Conclusions: Our results suggest that PTEN negatively regulates eosinophil survival, chemotaxis, and allergic inflammation. The pharmacological targeting of PTEN may be a new strategy to treat eosinophilic disorders.

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CUTTING EDGE ORAL ABSTRACTS

EOSINOPHIL GRANULES FUNCTION EXTRACELLULARLY AS RECEPTOR-MEDIATED SECRETORY ORGANELLES

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Background: The abundant intracytoplasmic eosinophil granules contain multiple preformed cationic and cytokine proteins whose secretion from within intact eosinophils is recognized as important to the roles of these leukocytes in innate immunity. In tissue sites of many eosinophil-associated diseases, intact extracellular, membrane-bound eosinophil granules have been demonstrated. However, neither functional roles nor consequences of free extracellular eosinophil granules have been delineated.

Objectives: We evaluated the capacity of initially intracellular eosinophil granules to function autonomously outside of eosinophils as cell-free, secretory-responsive organelles.

Methods: We studied secretory responses of eosinophil granules isolated by subcellular fractionation. Granule-enriched fractions were analyzed by electron microscopy and identified based on their content of eosinophil peroxidase activity (EPO), and immunoreactivity for lysosome-associated membrane proteins (LAMP) -2 and -3 (also known as CD63) and major basic protein. In functional studies, granules were stimulated with interferon (IFN)-γ or eotaxin and eosinophil cationic protein (ECP) was measured in the supernatant by ELISA. Receptor expression on granule membranes was evaluated by flow cytometry.

Results: Isolated granules secreted their proteins, including cytokines, ECP, EPO and β-hexosaminidase, in response to IFN-γ or eotaxin. By flow cytometry, granules expressed "extracellular" domains for the IFN-γ receptor α chain and the eotaxin CCR3 receptor. IFN-γ- and eotaxin-elicited ECP secretion was dose-dependently inhibited by genistein and pertussis toxin, respectively. SB203580, SB 202190 and calphostin C inhibited secretion elicited by both stimuli, whereas LY2924002 suppressed eotaxin-elicited ECP secretion and did not inhibit the response induced by IFN-γ. Furthermore, brefeldin A suppressed IFN-γ- and eotaxin-induced ECP release, implicating a role for vesiculo-membrane structures within cell-free eosinophil granules in mediating their secretory responses.

Conclusion: We're demonstrating for the first time that eosinophil intracytoplasmic granules respond, upon extrusion from eosinophils, to specific cytokine/chemokine stimuli via cognate granule membrane-expressed receptors to activate intragranular signaling pathways that elicit granule protein secretion. These findings reveal a distinct capacity for an intracellular organelle to function extracellularly and identify a novel capacity of eosinophils, after their lysis, to mediate inflammation and immunomodulation.

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CUTTING EDGE ORAL ABSTRACTS

IMMUNOMODULATORY AND PRO-FIBROTIC PROPERTIES OF EOSINOPHILS IN LATE STAGE *SCHISTOSOMA MANSONI* INFECTION

Caroline M. Percopo[1], Thirumalai Ramalingam[2], Allen W. Cheever[2], Mark S. Wilson[2], Kimberly D. Dyer[1], James J. Lee[3], Nancy A. Lee[3], Paul S. Foster[4], Thomas A. Wynn[2] and Helene F. Rosenberg[1]

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Background: In our previous study, we determined that the eosinophils had no impact on traditional measures of acute infection (number and size of granulomata, liver fibrosis, hepatotoxicity, worm burden and egg deposition) in mice challenged with the helminth parasite, *S. mansoni* using the two novel models of complete eosinophil ablation (ΔdblGATA and TgPHIL) (Swartz, Dyer *et al*., Blood 2005). Elevated levels of serum IL-5 (4-fold) were observed among infected TgPHIL and ΔdblGATA mice when compared to their infected wild-type counterparts.

Objectives: To extend our study of *S. mansoni* infection in eosinophil-deficient (IL-5 gene-deleted) and eosinophil-ablated ΔdblGATA, TgPHIL) mouse models at later time points, including measures of hepatic fibrosis, cellular recruitment, and differential cytokine expression.

Methods: All wild type and eosinophil-deficient mice were inoculated percutaneously with 40 cercariae of *S.mansoni*. Serum was collected at 4, 8, 12 and 16 weeks of infection. Mice were sacrificed at 16 weeks for an assessment of liver histopathology, cellular recruitment and for preparation of RNA. Serum levels of IFN-γ, TGF-β and IL-13RA2 were determined by ELISA. Quantitative PCR was performed to detect transcripts encoding IFN-γ, IL-13, and various markers of hepatic fibrosis.

Results: In contrast to our findings at 12 weeks, our results reveal significant differences in the levels of hydroxyproline per 10,000 eggs at 16 weeks of infection, which reflect differences in the degree of liver fibrosis, when comparing eosinophil-deficient mouse strains to their respective wild-type counterparts. We also observe augmented recruitment of mast cells, $CD4^+$T cells and IFN-γ^+CD4^+T cells, elevated expression of serum IL-13RA2, and increased expression of transcripts encoding IFN-γ among specific subsets of eosinophil-deficient mice.

Conclusions: Increases in IFN-γ transcription, $CD4^+$T cell recruitment and fluctuations in hepatic fibrosis suggest that eosinophils may have related roles in immunomodulation and tissue remodeling in later stages of infection with *S. mansoni*. Further study of these responses will further elucidate a definitive function for IL-5 and for eosinophils in the pathogenesis of parasitic helminth infection.

CUTTING EDGE ORAL ABSTRACTS

EOSINOPHILS ARE REQUIRED FOR ALLERGEN-INDUCED TH2 INFLAMMATION

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Background: The onset and progression of allergic asthma are characterized by Th2 inflammation and cellular infiltration of eosinophils into the lung tissues and airways. It has been hypothesized that eosinophils are recruited to the airways for their destructive effector functions in pulmonary inflammation. Our data suggest that in addition to these potential activities, eosinophils contribute to Th2-mediated pulmonary pathologies through modulation of the Th2 cytokines and T cell activation/recruitment

Objectives: To demonstrate the role of eosinophil immune regulation in Th2-mediated pulmonary inflammation.

Methods: Mice congenitally devoid of eosinophils (transgenic line: ***PHIL***) and wild type litter mates were sensitized and challenged with ovalbumin (OVA) and used as part of studies that included adoptive cell transfer to define the immune regulating role(s) of eosinophils contributing to allergen-mediated pulmonary pathologies.

Results: ***PHIL*** mice that undergo the OVA sensitization/challenge had reduced bronchoalveolar lavage (BAL) levels of Th2 cytokines (i.e., IL-4, -5, 13) as compared to wild type litter mates. Adoptive cell transfer studies using Th2 polarized OVA transgenic T cells (OT-II) demonstrated that Th2 polarized T cells were insufficient to induce wild type levels of Th2 cytokine production in the airways of eosinophil-less mice. Furthermore, adoptive transfer of eosinophils increased BAL Th2 cytokines levels and accumulation of T effector cells (CD62L(-)CD44(+)) in the lungs of allergen-challenged mice.

Conclusions: Eosinophil effector functions in pulmonary Th2 inflammation likely includes not only destructive effector functions, but also immune regulating roles. The data suggest eosinophils contribute to Th2 inflammation through direct and indirect modulation of Th2 cytokines. More importantly, eosinophils appear to enhance Th2 inflammation through the activation/recruitment of T cells to the lymph nodes and lungs of allergen sensitized and challenged mice, which in turn implicates an expanded role for eosinophils in the onset and maintenance of allergic asthma.

CUTTING EDGE ORAL ABSTRACTS

ROLE OF REGULATORY T CELLS IN EXPERIMENTAL EOSINOPHILIC ESOPHAGITIS (EE)

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Background: We recently showed a critical role for adaptive T cell immunity in the induction of EE and in this study now we identified specific lymphocyte subpopulations that are increased in experimental EE.

Objective: To examine activated T cells and regulatory T cells in experimental EE.

Methods: Experimental EE was induced in mice by intranasal *Aspergillus fumigatus* antigen treatment. Total cells from the mouse esophagus were isolated by tissue enzymatic digestion and flow cytometry (FACS) was performed to identify T-lymphocyte subpopulations.

Results: FACS analysis identified comparable levels of $CD3^+$ cells and $B220^+$ cells, increased levels of $CD4^+$ cells and decreased levels of $CD8^+$ cells in the esophagus of allergen-treated mice compared to saline-treated mice. Interestingly, we found an increase of activated lymphocyte subpopulations ($CD4^+CD25^+$, $CD4^+CD69^+$, $CD4^-CD25^+$, $CD4^-CD69^+$) and decreased regulatory T cells ($CD4^+CD45rb^{low}FOXP3^+$) in the esophagus. The absolute numbers of $CD4^+CD25^+$, $CD4^+CD69^+$, $CD4^+CD45rb^{low}FOXP3^+$, and $CD4^+CD45rb^{high}FOXP3^-$ are $7.1 \pm 0.5 \times 10^3$, $5.7 \pm 0.9 \times 10^3$, $8.4 \pm 2.0 \times 10^2$, and $6.1 \pm 1.7 \times 10^2$ in the esophagus of allergen-treated mice compared to $3.4 \pm 0.5 \times 10^3$, $3.1 \pm 0.3 \times 10^3$, $32.6 \pm 5.7 \times 10^2$ and $1.6 \pm 1.1 \times 10^2$ in the esophagus of saline-treated mice, respectively.

Conclusion: Allergen induced experimental EE is associated with the imbalance in pro-inflammatory cytokines producing ($CD4^+CD45rb^{low}FOXP3^+$) and anti-inflammatory cytokines producing ($CD4^+CD55rb^{high}FOXP3^-$) cells. Thus, indicates contributory role of regulatory T cells.

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CUTTING EDGE ORAL ABSTRACTS

HUMAN EOSINOPHILS IN ORAL SQUAMOUS CANCER

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Background: Eosinophils are the predominant inflammatory leukocyte infiltrating oral squamous carcinoma (OSC). However, little is known about the exact role eosinophils play in tumour regression, factors leading to their recruitment and prognostic value. Prostaglandins are known to be secreted by oral carcinomas and may be involved in specific eosinophil infiltration.

Objectives: To investigate how human eosinophils are specifically recruited to sites of selective tumour growth by prostaglandins and potentially play an anti-neoplastic role through the release of basic proteins.

Methods: Luna and MBP staining for eosinophils in surgically-resected primary oral squamous cancer was performed and correlated with tumor staging/grade and clinical outcome. Blood eosinophils were purified from eosinophilic subjects by immunomagnetic negative selection. The anti-neoplastic effect of eosinophils was determined by co-culture assay with SCC-9 OSC cell line. Eosinophil degranulation was evaluated by peroxidase (EPO) release colorimetric assay (OPD). Eosinophil chemotaxis toward OSC was measured by infiltration and migration through artificial basement membrane Matrigel™.

Results: Eosinophils infiltration was observed around tumour mass in our first cohort of 21 subjects. Eosinophil infiltration appeared to be more prominent in lower grade/stage OSC. Deposition of MBP granules is also observed in the surrounding tissue of tumor mass. We observed 40% growth inhibition of SCC-9 during co-culture with eosinophils for 72h. This growth inhibition correlated with EPO activity. SCC-9 induced strong transmigration of eosinophils (30±5%, $n = 14$, $p < 0.0001$) comparable to eotaxin control (38±6%). PGD_2 synthase inhibitor (HQL-79) abrogated migration toward SCC-9.

Conclusion: Our results suggest that eosinophils are selectively recruited by PGD_2 secreted by OSC and release basic proteins that possess very strong growth inhibiting activity in the surrounding of the tumor mass. These properties may be implicated in the positive prognosis associated with eosinophilic infiltration in OSC. These observations suggest modulating eosinophilia in certain types of cancers may have adjuvant immunotherapy potential.

CUTTING EDGE ORAL ABSTRACTS

EOSINOPHIL GRANULE MAJOR BASIC PROTEIN INDUCES APOPTOSIS AND INFLAMMATION OF BRONCHIAL EPITHELIAL CELLS INFECTED WITH RESPIRATORY SYNCYTIAL VIRUS

Masahiko Kato[1,2], Hirohito Kita[3], Taisei Ishioka[1], Hiroyuki Tsukagoshi[1], Kunihisa Kozawa[1], and Hirokazu Kimura[4]

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Background: Respiratory virus infection, such as rhino or respiratory syncytial virus (RSV), is an important exacerbatory factor in acute bronchial asthma. Further, RSV infection often produces the first episode of wheezing in children who go on to develop chronic asthma. However, the precise mechanisms responsible for viral infection-induced exacerbation of bronchial asthma are still uncertain.

Objectives: To evaluate the role of eosinophilic inflammation in the pathogenesis of virus-induced asthma, we investigated the effects of major basic protein (MBP) and other eosinophilic granule proteins on bronchial epithelial cells infected with RSV.

Methods: Morphological changes and cytopathic effects (CPE) in human type II pulmonary alveolar epithelial cells (A549) cells infected with RSV (multiplicity of infection (MOI) of 0.1, 1.0, and 10) and/or MBP at several concentrations were observed by microscopy. Apoptosis of A549 cells was evaluated by flow cytometric analysis using annexin V and propidium iodide staining, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, we measured 17 types of cytokines and chemokines in supernatants from A549 cells treated with RSV and/or MBP, and measured 8 types of phosphorylation of intracellular signaling molecules from A549 cells using a multiplex beads-based assay (Bio-Rad).

Results: Although RSV alone did not affect CPE or apoptosis of A549 cells, high concentrations (> 25 μg/ml) of MBP resulted in CPE within 24 hours. Combinations of RSV and MBP synergistically induced cytotoxicity and apoptosis of cells. This effect was abolished by ultraviolet (UV) treatment, suggesting necessity of a live virus. In A549 cells treated with MBP alone, production of interleukin (IL)-2, 4, 5, 7, 10, 12, 13, 17, interferon (IFN)-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, and macrophage inflammatory protein (MIP)-1β was significantly increased in comparison with treatment with RSV alone. Further, levels of GM-CSF and IL-17 in cells treated with RSV/MBP were significantly greater than with RSV or MBP alone. Finally, the phosphorylation of intracellular signaling molecules, including ATF-2, p38 MAPK, Akt, and GSK-3α/β, was increased by treatment with RSV/MBP compared with RSV or MBP alone.

Conclusions: These results suggest that MBP synergistically enhanced apoptosis and inflammation of RSV-infected epithelial cells, indicating that eosinophilic inflammation may be closely associated with the pathophysiology of acute exacerbation of bronchial asthma.

CUTTING EDGE ORAL ABSTRACTS

CONTRIBUTION OF TNF-α TO THE EXQUISITE ADAPTABILITY OF THE EOSINOPHIL TO INFLUENCE INFLAMMATION AND REPAIR

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Background: Eosinophils (EOS) are capable of expressing numerous factors that influence inflammation and tissue repair/remodeling. In the presence of a single stimulus, however, most of these factors are only detectable at the mRNA level or at minute protein concentrations. A protein array (120 proteins) analysis of human EOS stimulated with Th1 (IFN-γ), Th2 (IL-4), or IL-5 family (IL-3, IL-5, GM-CSF) cytokines alone or in combination with the EOS-priming cytokine TNF-α revealed that TNF-α provided a potent synergistic signal for EOS generation of substantial amounts of Th1 chemokines (IP-10/MIG), Th2 chemokines (TARC/MDC), and the matrix metalloproteinase MMP-9.

Objectives: The aim of this study was to explore the mechanisms behind the synergistic effect of TNF-α on EOS generation of Th1/Th2 chemokines and MMP-9.

Methods: Highly purified (negative selection with anti-CD16, -CD14, and -CD3) human blood EOS were cultured with IFN-γ, IL-4, IL-3, IL-5, or GM-CSF alone or in combination with TNF-α. Concentrations of Th1 chemokines, Th2 chemokines, and MMP-9 in EOS culture supernatant fluids were measured by ELISA, mRNA expression by quantitative real-time PCR, and signaling events by intracellular flow cytometry.

Results: When highly purified, human EOS produced little, if any Th1/Th2 chemokines or MMP-9 in response to a single cytokine. Generation of Th1 and Th2 chemokines was synergistically induced by TNF-α plus IFN-γ or IL-4 respectively, but not by TNF-α plus IL-5 family cytokines. TNF-α with IFN-γ or IL-4 had little effect on EOS MMP-9 release, but very high levels were produced in the presence of TNF-α plus IL-5 family cytokines (with IL-3>>GM-CSF>IL-5). The synergistic effect of TNF-α for IFN-γ-induced IP-10/MIG, IL-4-induced TARC/MDC, and IL-3-induced MMP-9 occurred both at the protein and mRNA levels. While activation of NF-κB, STAT1, STAT3, and STAT6 was induced in EOS by TNF-α, IFN-γ, IL-3, and IL-4 respectively, there was no evidence for enhancement of these signaling events when cytokines were added in combination. Utilization of pharmacological inhibitors revealed that activation of NF-κB appears critical for EOS generation of IP-10, MIG, TARC, MDC, and MMP-9; whereas inhibitors of the MAP kinases ERK1/2, p38, or JNK, only partially decreased Th1/Th2 chemokine or MMP-9 production in response to TNF-α plus the respective activators.

Conclusions: TNF-α, working, at least in part, through the activation of NF-κB, serves as a critical co-activator for EOS production of Th1/Th2 chemokines and MMP-9. These data suggest that EOS are exquisitely adaptable to cytokines present in the microenvironment and that TNF-α contributes to the EOS ability to influence inflammation and tissue repair/remodeling.

This project was funded in part by NIH grants HL56396 and M01RR03186.

CUTTING EDGE ORAL ABSTRACTS

IDENTIFICATION AND IMPROVED DIAGNOSIS OF EOSINOPHIL ESOPHAGITIS PATIENTS FROM ARCHIVED CLINICAL BIOPSIES USING A NOVEL ANTI-EOSINOPHIL PEROXIDASE MONOCLONAL ANTIBODY

Cheryl Protheroe, Giovanni De Petris, Shabana Pasha, Virender Sharma, Tisha N. Lunsford,Lucinda Harris, Michael P. McGarry, Nancy A. Lee, and James J. Lee Mayo Clinic Arizona, Scottsdale, AZ.

Rationale: Traditional pathologist-driven identification and diagnosis of eosinophil esophagitis (EE) patients is achieved by time consuming examination of patient biopsies at high (40X) magnification (hpf) to identify the presence of at least a single foci of ≥ 15 eosinophils. Thus, this diagnostic strategy is strictly qualitative (i.e., a patient either has or doesn't have the required foci of ≥ 15 eosinophils) and fails to include any measure of eosinophil activation leading to the release of granule proteins (i.e., degranulation). Unfortunately, the lack of functionally sensitive eosinophil-specific antibodies useful in immunohistochemistry (IHC) platforms have prevented the development of improved methodologies that are reproducibly more sensitive and quantitative.

Methods: We have developed a novel anti-eosinophil peroxidase (EPX) mouse monoclonal antibody by sensitizing EPX knockout mice with purified eosinophil peroxidase. This antibody is capable of reliably detecting both tissue eosinophils and evidence of released EPX using the most common archived clinical samples - formalin-fixed paraffin-embedded tissues.

Results: We have developed a standardized anti-EPX based IHC assay to create a new quantitative diagnostic criteria for the identification of EE patients. In particular, this new algorithm includes not only a determination of intact tissue eosinophil density throughout the available biopsies but also a unique assessment of the level of eosinophil activation/degranulation. Our study examines biopsies from EE and GERD patients who were diagnosed on the basis of the currently accepted pathologist-driven definition of the presence of at least one foci containing ≥ 15 eosinophils/hpf from biopsies >10cm from the esophageal-gastric junction. This new IHC assay confirmed that in many of these cases the presence of intact eosinophils alone was insufficient to achieve a definitive diagnosis of EE. In contrast, our EPX-based algorithm was shown not only to be a remarkably reliable measure of intact eosinophils throughout the available biopsies of a given patient but also revealed evidence for extensive and often regionally-specific eosinophil degranulation in these patients. That is, in addition to the ability to quantify intact eosinophil density, this assay was capable of detecting and visualizing the presence of both enucleated cytoplasmic fragments and large numbers of individual free eosinophil secondary granules in biopsy specimens that sometimes failed to achieve the "gold standard" of ≥ 15 eosinophils/hpf.

Conclusions: The availability of this new anti-EPX monoclonal antibody-based assay provides the pathology community with a reliably sensitive and quantitative algorithm for the identification and diagnosis of patients with eosinophil-associated diseases including EE.

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CUTTING EDGE ORAL ABSTRACTS

EX VIVO DIFFERENTIATION OF EOSINOPHILS FROM THE ΔDBLGATA MOUSE

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Background: Ablation of the dblGATA enhancer site that regulates expression of GATA-1 results in the eosinophil-ablated phenotype of the ΔdblGATA mouse (Yu et al., J Exp Med 2002).

Objectives: To understand the molecular events that result from the ablation of the dblGATA enhancer site and to gain increased appreciation for the critical events in eosinophil hematopoiesis.

Methods: *Ex vivo* culture of mouse bone marrow in the presence of SCF, FLT3L, IL5, IL3 and GMCSF. Analysis of transcript expression by quantitative RT-PCR; enumeration of eosinophil count by staining cells with anti-Siglec F and analysis by flow cytometry; analysis of protein expression by staining with anti-MBP followed by imaging with confocal microscopy; analysis of specific GATA-1 promoter use by 5' RACE followed by sequencing and mapping onto known gene structure.

Results: We find that bone marrow progenitors isolated from ΔdblGATA mice can differentiate into mature eosinophils *ex vivo*. Cultured ΔdblGATA eosinophils contain cytoplasmic granules with immunoreactive major basic protein and they express surface Siglec F and transcripts encoding major basic protein, eosinophil peroxidase, and GATA 1, 2, and 3 to an extent indistinguishable from cultured wild type progenitors. The eosinophil deficit observed *in vivo* is an intrinsic progenitor defect, and remains unaffected by interactions with stromal cells. Donor wild-type bone marrow transplants produce eosinophils in ΔdblGATA recipient mice while ΔdblGATA bone marrow failed to repopulate the eosinophil fraction in wild-type recipients. At the molecular level, 45% of the transcripts encoding GATA-1 detected in cultured ΔdblGATA progenitors express a variant transcript that includes a first, proximal exon $1E_B$ in place of the characterized distal hematopoietic exon $1E_A$, which is the predominant form of GATA-1 transcript (83%) detected in wild-type cultures ($p < 0.001$) Interestingly, most of the GATA-1 transcripts detected in freshly-isolated bone marrow progenitors from ΔdblGATA mice include the $1E_A$ exon ($p<0.001$ vs. cultured ΔdblGATA progenitors). In contrast, the distribution of GATA-1 transcripts from wild-type bone marrow is not altered significantly in response to culture conditions.

Conclusions: These data suggest that cultured progenitors are able to circumvent the effects of the ΔdblGATA ablation by utilizing the second, more proximal $1E_B$ promoter, and may use this mechanism to generate quantities of GATA-1 that will support eosinophil growth and differentiation *ex vivo*.

CUTTING EDGE ORAL ABSTRACTS

MEDI-563 IS A HUMANIZED ANTI-HUMAN IL-5Rα ANTIBODY WITH ENHANCED EFFECTOR FUNCTION

Alexander Kozhich[1], Jennifer L. Reed[1], Jonas S. Erjefalt[2], Cecilia Andersson[2], Bonnie Swerdlow[1], Nanci Donacki[1], Peter Kiener[1], Alison A. Humbles[1], Rob Woods[1], Anthony J. Coyle[1] and Roland Kolbeck[1]

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Background: The suboptimal efficacy of IL-5-targeted therapies in asthma has been attributed to an incomplete depletion of eosinophils in lung tissue. MEDI-563 is a fully humanized anti-human IL-5Rα antibody which not only inhibits the productive interaction of IL-5 with its high affinity receptor, but also depletes IL-5Rα expressing cells through enhanced ADCC activity. Quantitative depletion of tissue eosinophils and other IL-5Rα expressing effector cells (basophils, mast cells) by MEDI-563 might result in enhanced clinical efficacy.

Objectives: (1) To investigate the mechanism of action and potency of MEDI-563. (2) To study IL-5Rα expressing cell types and numbers in tissues of normal individuals and those with asthma.

Methods: MEDI-563 binding affinities to Fc-receptors and IL-5Rα was determined by surface plasmon resonance. Depletion of IL-5Rα expressing target cells *in vitro* was assessed using flow-cytometry and by measuring LDH in cell culture supernatants. MEDI-563 and commercially available antibodies were used to assess IL-5Rα cell surface expression in blood and in tissues by flow cytometry and immunohistochemistry, respectively.

Results: MEDI-563 bound hIL-5Rα with an affinity of 2.1nM and in ADCC assays lysed IL-5Rα transfected cells *in vitro* with an EC50 of ~0.06nM, whereas a a-fucosylated isotype-control antibody was completely ineffective and the fucosylated form of MEDI-563 showed significantly lower potency. The enhanced ADCC of MEDI-563 correlated with a 10-fold higher binding affinity to human FcgRIIIa (K_D=46nM) in comparison to a wild-type isotype control (K_D=574nM). However, binding affinities of MEDI-563 and the wild-type control antibody to human Fc receptors FcgRI, FcgRIIa and FcgRIIb were nearly indistinguishable. In peripheral blood IL-5Rα expression is restricted to eosinophils and basophils. The potency of MEDI-563 to effectively deplete eosinophils, basophils and mast cells and its correlation with cell surface expression of IL-5Rα is the focus of ongoing studies. We were able to show IL-5Rα expression on a subset of eosinophils in nasal polyps and on mast cells in lungs of IL-9 transgenic mice. We are currently expanding our efforts to study cell types and numbers expressing IL-5Rα in bronchial biopsies and bone-marrow aspirates from normal and asthmatic individuals.

Conclusions: MEDI-563 more effectively depletes IL-5Rα expressing target cells through enhanced ADCC *in vitro*.

Funded by MedImmune, Inc and Biowa, Inc.

CUTTING EDGE ORAL ABSTRACTS

MEDI-563, AN ANTI-INTERLEUKIN-5-RECEPTOR ANTIBODY, IS WELL TOLERATED AND INDUCES REVERSIBLE BLOOD EOSINOPENIA IN MILD ASTHMATICS IN A PHASE I TRIAL, MI-CP-158

William W. Busse[1], Rohit Katial[2], Francisco Leon[3], Anthony J. Coyle[3], George L. Spitalny[4], Masamichi Koike[4], Alistair Wheeler[4], Nobuo Hanai[4] and Barbara White[3]

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Background: Eosinophils are believed to play a key role in the pathogenesis of asthma. Interleukin-5 (IL-5) is a major cytokine in eosinophil biology, and expression of its receptor (IL-5R) is largely restricted to eosinophils, basophils, and mast cells. The suboptimal efficacy of IL-5-targeted therapies in asthma has been attributed to an incomplete depletion of eosinophils in lung tissue. Complete lung eosinophil depletion should provide additional insight into the role of these cells in asthma and could represent a novel therapeutic strategy.

Objectives: To assess the safety and biological activity of MEDI-563 (previously known as BIW-8405), a humanized afucosylated IgG1 anti-IL-5R α chain monoclonal antibody. MEDI-563 was developed by BioWa, Inc. through proprietary Potelligent® technology that significantly enhances antibody-dependent cellular cytotoxicity. MEDI-563 neutralizes IL-5 activity and depletes tissue eosinophils in pre-clinical models with an acceptable toxicology profile.

Methods: Six subjects with mild asthma and absence of corticosteroid therapy were enrolled in the first cohort of study MI-CP-158 (also known as BIW-8405-001), an open-label first-in-human study with MEDI-563. The patients received a single intravenous dose of 0.03 mg/kg MEDI-563 and were followed for 84 days.

Results: MEDI-563 was well tolerated, and no serious adverse events were reported. All adverse events (AEs) were mild, and the most frequently reported AE was fatigue on the dosing day post administration (3/6 subjects). Circulating eosinophils decreased below detection limits within 24-48 hours of dosing in all 6 subjects, from 4.4±2.6% (232±96 cells /mm^3) at baseline. This effect lasted for 8-12 weeks, and eosinophils became detectable in some subjects at Day 58 post dosing and reached ≥70% of baseline levels by Day 84 post dosing in 4/5 subjects analyzed. Circulating basophils followed an overall similar trend. MEDI-563 administration was associated with immediate (within 6 hrs), modest (<10x baseline) and transient (<1 week duration) increases in serum C-reactive protein (2/6 subjects) and IL-6 (3/5).

Conclusions: A single 0.03 mg/kg IV dose of MEDI-563 induces a robust blood eosinopenia, with an acceptable safety profile to date. These findings support further exploration of MEDI-563 as a potential therapy for eosinophil-mediated diseases.

Funded by MedImmune, Inc. and Biowa, Inc.

POSTER DISCUSSION SESSION I

Cell Biology and Eosinophil Studies (Posters 1-29)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
1	**Bedi,** Richa	TO BE OR NOT TO BE AN EOSINOPHIL – ROLE OF HUMAN CCAAT/ENHANCER BINDING PROTEIN EPSILON (C/EBPε) ISOFORMS	Richa Bedi and Steven J. Ackerman
2	**Wagner,** Lori	EGO, A NOVEL, NON-CODING RNA GENE, REGULATES EDN AND MBP mRNA LEVELS DURING EOSINOPHIL DEVELOPMENT	Lori A. Wagner, Clarissa J. Christensen, Diane M. Dunn, Gerald J. Spangrude, Ann Georgelas, Linda Kelley, M. Sean Esplin, Robert B. Weiss and Gerald J. Gleich
3	**Moser,** Jennifer	THE ROLE OF PERP, A DOWNSTREAM EFFECTOR OF THE TUMOR SUPPRESSOR P53 AND ITS HOMOLOGUE P63, IN EOSINOPHIL HEMATOPOIESIS	Jennifer M. Moser, Kimberly D. Dyer, Helene F. Rosenberg
4	**Qiu,** Zhijun	GATA-1 AND GATA-2 REGULATE TRANSCRIPTION OF EOSINOPHIL-DERIVED NEUROTOXIN (EDN) AND EOSINOPHIL CATIONIC PROTEIN (ECP) IN HUMAN EOSINOPHILS	Zhijun Qiu, Kimberly D. Dyer and Helene F. Rosenberg
5	**Spencer,** Lisa	HUMAN EOSINOPHILS EXPRESS FUNCTIONAL NOTCH LIGANDS	Lisa A. Spencer, Lauren Reynolds and Peter F. Weller
6	**Melo,** Rossana	VESICULAR TRANSPORT OF MAJOR BASIC PROTEIN (MBP) WITHIN ACTIVATED HUMAN EOSINOPHILS	Rossana C. N. Melo, Lisa A. Spencer, Sandra A. C. Perez, Staci P. Bafford, Ann M. Dvorak and Peter F. Weller
7	**Ellis,** Anne	MOLECULAR BIO-MARKERS OF EOSINOPHIL-LINEAGE COMMITMENT: MULTIPLEX Q-PCR ANALYSIS OF GATA-1, MBP AND IL-5 RECEPTOR MRNA EXPRESSION KINETICS	Anne K. Ellis, Lynn Crawford, and Judah A. Denburg
8	**Langlois,** Anick	REGULATION OF MMP-9 RELEASE BY HUMAN EOSINOPHIL MIGRATION	A. Langlois, F. Chouinard, C. Ferland, and M. Laviolette
9	**Bracke,** Madelon	TEMPORAL DIFFERENCES IN CORTICOSTEROID EFFECTS ON OPSONIN AND ADHESION RECEPTORS ON HUMAN EOSINOPHILS	Willem ten Hove, Leo A. Houben, René C. Schweizer, Hubertus G.M. Leufkens, Jan A.M. Raaijmakers, Leo Koenderman and Madelon Bracke
10	**Takaku,** Yotaro	EOSINOPHIL FUNCTIONS INDUCED BY ADHESION MOLECULES AND LEUKOTRIENE D4	Yotaro Takaku, Mariko Mori, Takehito Kobayashi, Koichi Hagiwara, Minoru Kanazawa, and Makoto Nagata

POSTER DISCUSSION SESSION I

Cell Biology and Eosinophil Studies (Posters 1-29)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
11	**Adamko,** Darryl	THE EFFECT OF CATIONIC CHARGE ON RELEASE OF EOSINOPHOL MEDIATORS:ROLE OF SURFACE RECEPTORS	Darryl J. Adamko, Yingqi Wu, Farnam Ajamian, Redwan Moqbel, and Gerald J. Gleich
12	**Andersson,** Kerstin	HOUSE DUST MITE ALLERGEN ACTIVATES HUMAN EOSINOPHILS VIA FORMYL PEPTIDE RECEPTOR AND FORMYL PEPTIDE RECEPTOR-LIKE 1	Kerstin Andersson, Lena Svensson, Elin Redvall, Camilla Björn, Jennie Karlsson, Ann-Marie Bergin, Marie-Josèphe Rabiet, Claes Dahlgren and Christine Wennerås
13	**Coughlin,** Jason	RAB27A IN HUMAN EOSINOPHILS: DIFFERENTIAL ACTIVATION PATTERNS	Jason J. Coughlin and Redwan Moqbel
14	**Dombrowicz,** David	DIRECT MYCOBACTERIUM BOVIS-BCG RECOGNITION BY HUMAN EOSINOPHILS INVOLVES TOLL-LIKE RECEPTOR 2 AND TRIGGERS DELTA-DEFENSIN PRODUCTION	Virginie Driss, Fanny Legrand, Emmanuel Hermann, Sylvie Loiseau, Yann Guerardel, Laurent Kremer, David Dombrowicz and Monique Capron
15	**Doyle,** Christine	IDENTIFICATION OF INTRACELLULAR LIGANDS FOR HUMAN GALECTIN-10 (CHARCOT-LEYDEN CRYSTAL PROTEIN) IN EOSINOPHILS: NOVEL INTERACTIONS WITH GRANULE CATIONIC RIBONUCLEASES	Christine B. Doyle, Li Liu and Steven J. Ackerman
16	**Lee,** Vicki	EOSINOPHIL-DERIVED NEUROTOXIN (EDN) AND EOSINOPHIL CATIONIC PROTEIN (ECP) AS POTENTIAL ENDOGENOUS LIGANDS OF THE TOLL-LIKE RECEPTOR-2 SIGNAL TRANSDUCTION PATHWAY	Vickie K. Lee, Kimberly D. Dyer, De Yang and Helene F. Rosenberg
17	**Hamada,** Kazuyuki	A HIGH RF LEVEL INDICATES EOSINOPHIL ACTIVATION, AND IS A CLINICAL MARKER OF SEVERITY OF ASTHMA	Kazuyuki Hamada, Yoshiki Kobayashi and Hideo Kita
18	**Koziol,** Cynthia	PEPTIDE-NUCLEIC ACID-MEDIATED KNOCKDOWN OF THE IL-5-DEPENDENT GENE, PIM-1, IN HUMAN PERIPHERAL BLOOD EOSINOPHILS LEADS TO A DECREASE IN IL-5-MEDIATED CELL SURVIVAL	Cynthia J. Koziol, Mary E. Bates and Paul J. Bertics
19	**Ueki,** Shigeharu	RETINOIC ACIDS INDUCE SURVIVAL AND CYTOKINE PRODUCTION IN EOSINOPHILS	Shigeharu Ueki, Gulixian Mahemuti, Hikari Kato, Miyoshi Fujita, Hajime Oyamada, Yumiko Kamada, Masahide Takeda, Kazutoshi Yamaguchi, Takahito Chiba, Wataru Ito, Hiroyuki Kayaba and Junichi Chihara

POSTER DISCUSSION SESSION I

Cell Biology and Eosinophil Studies (Posters 1-29)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
20	**Takeda,** Masahide	PROCATEROL ENHANCES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)Γ EXPRESSION IN HUMAN EOSINOPHILS THROUGH BETA2-ADRENOCEPTOR	Shigeharu Ueki, Masahide Takeda, Atsuko Usami, Hajime Oyamada, Miyoshi Fujita, Wataru Ito, Hikari Kato, Tomomi Tanigai, Takahito Chiba, Kazutoshi Yamaguchi, Hiroyuki Kayaba and Junichi Chihara
21	**Bandeira-Melo,** Christianne	PGD2 ELICITS DIFFERENTIAL RELEASE OF PRE-FORMED CYTOKINES FROM EOSINOPHILS: COOPERATIVE SIGNALING BETWEEN DP AND CRTH2 RECEPTORS	Christianne Bandeira-Melo, Mesquita-Santos, Bozza, P.T. and Diaz, B.L.
22	**Chouinard,** Francois	MONTELUKAST REGULATION OF CYSTEINYL LEUKOTRIENE RELEASE BY BLOOD EOSINOPHILS	F. Chouinard, A. Langlois, N. Flamand, C. Ferland and M. Laviolette
23	**Morgan,** Paul	RESVERATROL INHIBITS EOSINOPHIL PEROXIDASE-INDUCED MAST CELL DEGRANULATION	Paul M. Morgan, Agnes Ciarletta and Marion Kasaian
24	**Odemuyiwa,** Solomon	TRYPTOPHAN CATABOLITES MODULATE EOSINOPHIL DEGRANULATION THROUGH GLUTAMATE RECEPTORS	Solomon O. (Wole) Odemuyiwa, Mazyar Ghaffari, Victoria Lam, Darryl Adamko1 Carina Majaesic, and Redwan Moqbel
25	**Eriksson,** Jenny	A SELDI-TOF MS STUDY OF THE MOLECULAR HETEROGENEITY OF EOSINOPHIL CATIONIC PROTEIN	Jenny Eriksson, Charlotte Woschnagg, Eva Fernvik and Per Venge
26	**Torrent,** M	EOSINOPHIL CATIONIC PROTEIN (ECP) ACTION AT THE BACTERIA CELL WALL AND CYTOPLASMIC MEMBRANE	Torrent M., Sánchez, D., Moussaoui, M., Navarro, S., Nogués M.V. and Boix E.
27	**Blanchard,** Carine	EOSINOPHILIC ESOPHAGITIS IS MEDIATED BY A CORTICOSTEROID REVERSIBLE IL-13-STIMULATED KERATINOCYTE TRANSCRIPTOME INVOLVING EOTAXIN-3	Carine Blanchard, Melissa K. Mingler, Margaret H. Collins, Philip E. Putnam and Marc E. Rothenberg
28	**Legrand,** Fanny	EOSINOPHILS: NEW EFFECTORS IN TUMOR IMMUNITY	Fanny Legrand, Virginie Driss, Emmanuel Hermann, Sylvie Loiseau, Jean-Emmanuel Kahn, Lionel Prin, David Dombrowicz and Monique Capron
29	**Ofosu-Appiah,** William	GENERATION AND CHARACTERIZATION OF POTELLIGENT® ANTI-HUMAN IL-5R-ALPHA CHAIN HUMANIZED MONOCLONAL ANTIBODY, MEDI 563	Masamichi Koike, Kazuyasu Nakamura, Akiko Furuya, Emi Shoji-Hosaka, Akihiro Iida, Hideharu Anazawa, Kenya Shitara, Kiyoshi Takatsu and Nobuo Hanai

POSTER DISCUSSION SESSION I

POSTER #1

TO BE OR NOT TO BE AN EOSINOPHIL – ROLE OF HUMAN CCAAT/ENHANCER BINDING PROTEIN EPSILON (C/EBPε) ISOFORMS

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Background: C/EBPε is required for the terminal differentiation of granulocytes, both eosinophils and neutrophils. Human C/EBPε is expressed as four distinct isoforms (32, 30, 27, 14kD) through alternative promoters, RNA splicing, and translational start sites. The C/EBP$\varepsilon^{32/30}$ isoforms function as transcriptional activators. The C/EBPε^{27} isoform interacts with and is a repressor of GATA-1 transactivation of eosinophil genes such as MBP1. C/EBPε^{14}, which lacks a transactivation domain, may function as a dominant negative repressor of C/EBP$\varepsilon^{32/30}$ or other C/EBPs required for eosinophil development.

Objectives: To study the activities of the C/EBPε isoforms in cord blood (CB) CD34+ progenitors to define their roles in lineage specification and eosinophil differentiation.

Methods: We assessed mRNA expression for the C/EBPε isoforms using semi-quantitative RT-PCR during eosinophil differentiation of CB CD34+ progenitors using SCF, IL-3, and IL-5. To study the activities of the C/EBPε isoforms, we subcloned their cDNAs into an MSCV-based bicistronic retroviral vector (pGCDNsam IRES-EGFP) and ectopic expression was induced in CB CD34+ progenitors by retroviral transduction for 72hrs. The CD34+/GFP+ cells were sorted by FACS and plated in semi-solid colony assay media (Collagen Cult™) containing SCF, IL-3 and a lineage-specific cytokine (i.e. erythropoietin (EPO), G-CSF, or IL-5), or in suspension culture containing SCF, IL-3 and IL-5 to drive eosinophil differentiation. Differential colony and cell counts were performed after 15-17 days based on morphology, histochemical and enzyme staining.

Results: CD34+ cells initially express only the C/EBPε^{14} isoform, with induction of all isoforms by day 3. Results defined novel temporal changes in the expression ratios for the activator vs. repressor C/EBPε isoforms during eosinophil differentiation. The C/EBPε^{32} isoform altered CD34 progenitor development, strongly favoring eosinophil (>90%) over neutrophil or erythroid development, even in cultures containing G-CSF or EPO. C/EBPε^{27} inhibited both erythroid and eosinophil colony growth and increased granulocyte-macrophage colonies compared to C/EBPε^{14} or empty vector. C/EBPε^{14} increased erythroid colonies in myeloid conditions and inhibited eosinophil colony formation. Greater than 90% of cells transduced with C/EBPε^{32} grown in suspension culture were eosinophils, whereas C/EBPε^{27} and C/EBPε^{14} strongly inhibited eosinophil differentiation.

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

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POSTER DISCUSSION SESSION I

POSTER #2

EGO, A NOVEL, NON-CODING RNA GENE, REGULATES EDN AND MBP MRNA LEVELS DURING EOSINOPHIL DEVELOPMENT

Wagner, Lori A.[1], Christensen, Clarissa J.[1], Dunn, Diane M.[2], Spangrude, Gerald J.[3], Georgelas, Ann [1], Kelley, Linda[3], Esplin, M. Sean[5], Weiss, Robert B.[2], and Gleich, Gerald J[1,3]

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Background: The interactions of several transcription factors, including *GATA-1*, *PU.1*, and the CCAAT enhancer binding proteins, *c/EBP*α and ε, are involved in eosinophil development. High levels of *PU.1* direct myeloid differentiation by antagonizing *GATA-1* in the early stages of stem cell commitment. During later stages of eosinophil development, an intermediate level of *GATA-1* in synergy with *PU.1* directs the formation of the eosinophil lineage by activating dual binding sites in the *MBP* promoter. The CCAAT enhancer binding protein, *c/EBP*α, is important in early myeloid development, whereas *c/EBP*ε plays a later role.

Objectives: To identify new genes involved in eosinophil development.

Methods: Gene expression profiling of IL-5 stimulated CD34+ cord blood cells was performed to identify genes involved in eosinophil development. A novel gene, EGO, was identified by this method and gene expression was characterized. RNA silencing was also performed to determine the function of the gene.

Results: Gene expression profiling of early eosinophil development shows increased transcript levels of pro-inflammatory cytokines, chemokines, transcription factors and a novel gene, *EGO* (Eosinophil Granule Ontogeny). *EGO* is nested within an intron of the inositol triphosphate receptor type 1 (*ITPR1)* gene and is conserved at the nucleotide level; however, no large open reading frames are present. Sucrose density gradients show that *EGO* is not associated with ribosomes and therefore, is an untranslated RNA. *EGO* transcript levels increase 6 hours following interleukin-5 (IL-5) stimulation of $CD34^+$ hematopoietic progenitors. *EGO* RNA is also highly expressed and inducible in mature eosinophils. RNA silencing of *EGO* results in decreased major basic protein and eosinophil derived neurotoxin mRNA expression in developing $CD34^+$ hematopoietic progenitors *in vitro* and in a $CD34^+$ cell line model.

Conclusions: Therefore, *EGO* is a novel ncRNA gene expressed during development and is necessary for normal *MBP* and *EDN* transcript expression.

This project was funded by a grant from NIH, AI061097-01A1.

POSTER DISCUSSION SESSION I

POSTER #3

THE ROLE OF *PERP*, A DOWNSTREAM EFFECTOR OF THE TUMOR SUPPRESSOR p53 AND ITS HOMOLOGUE p63, IN EOSINOPHIL HEMATOPOIESIS

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Background: The study of eosinophil development and differentiation has profound implications toward the understanding and treatment of allergic disorders. Molecular events underlying the commitment and differentiation of eosinophil progenitors remain unclear. Microarray analysis comparing bone marrow from IL-5 gene-deleted (C57BL/6), and eosinophil-ablated _údblGATA (BALB/c) and TgPHIL (C57BL/6) mice and their appropriate wild-types both at baseline and in response to Th2 cytokine stimulation by *S. mansoni* infection revealed that the transcript encoding *Perp* (p53 apoptosis effector related to PMP-22 protein) was differentially regulated in all wild-type vs. all eosinophil-deficient/ablated mouse models. After Th2 stimulation, expression of *Perp* in TgPhil, ?_dblGATA, and IL-5-/- mice is 3.5, 4.7, and 6.5 fold higher, respectively, than their wild-type counterparts. *Perp* expression has not been reported in eosinophils or among cells of the granulocyte lineage. Results to date indicate that *Perp* is a mediator of p53-dependent apoptosis in thymocytes and neurons. *Perp* is likewise a target of p63 and is essential for epithelial integrity in mice and paradoxically, plays an anti-apoptotic role in the developing skin and notochord of zebrafish.

Objectives: Given its association with eosinophil lineage commitment and differentiation, we are exploring *Perp* expression as a part of the development of eosinophilic leukocytes in culture. We hypothesize that, as a cell-cycle modulator, *Perp* expression results in diminished replication of eosinophil progenitors and is intimately involved with pathways that result in commitment and acquisition of lineage-specific traits.

Methods: Short hairpin RNAs complementary to *Perp* mRNA were designed for packaging into lentiviral constructs, which has the demonstrated ability to infect and transfer genetic material successfully to the host cells, including bone marrow. qPCR and Western blot analysis will be used to determine the levels of *Perp* transcript levels and *Perp* protein in the cells.

Results: We have successfully generated a bone marrow culture system that demonstrates eosinophil development and *Perp* expression in wild-type bone marrow cells over a period of two weeks and faithfully reproduces the gene microarray findings determined *in vivo*. We have also generated lentiviral $Perp^{shRNA}$ constructs that successfully reduce *Perp* expression *in vitro*, and we are in the process of exploring the impact of *Perp* knockdown in mouse bone marrow cultures.

Conclusions: We will describe the effects of *Perp* knockdown, including cell phenotype, eosinophil number, and identification of downstream effectors. The experiments outlined include global analysis of genes in the apoptosis pathways, and the resultant cell culture phenotype will provide insight into the sufficiency and necessity of *Perp* in eosinophil hematopoiesis.

POSTER DISCUSSION SESSION I

POSTER #4

GATA-1 AND GATA-2 REGULATE TRANSCRIPTION OF EOSINOPHIL-DERIVED NEUROTOXIN (EDN) AND EOSINOPHIL CATIONIC PROTEIN (ECP) IN HUMAN EOSINOPHILS

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Background: Eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) are both RNase A superfamily ribonucleases that are stored in and ultimately secreted from the specific granules of activated human eosinophils. EDN and ECP are closely related genes (89% nucleotide sequence homology) with shared gene structure although with divergent expression patterns. Specific sequences within the single intron in both EDN and ECP genes plays an important role in regulating their transcription, including functional enhancer binding sites for NFAT-1 and PU.I in the gene encoding EDN and an NFAT-1 binding site in the gene encoding ECP. Additionally, a C/EBP binding site located at -124 in the 5' promoter of the EDN gene has been identified as involved in the regulation of transcription. However, the more distal 5' regions of both of the genes have not yet been explored.

Objective: To explore the impact of the distal 5' regions on the differential transcription of the genes encoding EDN and ECP.

Methods: For this study, 0.5, 0.8 and 1.4 kb of fragments including sequence directly 5' to the introns within the genes encoding EDN and ECP were amplified from the genomic DNA and subcloned into pGL3 reporter vector. Regulatory activity was assessed by luciferase assay of tranfectants in the eosinophil promyelocyte butyric-acid differentiated clone 15 cell line. MatInspector program available at www.genomatix.de was employed to determine consensus transcription factor binding sites, followed by verification through mutagenesis studies and electrophoretic mobility shift and supershift assays (EMSAs). Quantitative PCR and western blotting were used to determine quantitative changes in expression of transcription factors as well as EDN and ECP in response to differentiation-inducing stimuli.

Results: Luciferase activity of the 1.4 kb EDN 5' promoter fragment was about 2-fold greater than that of 0.5 and 0.8 kb promoter fragments. Given the 92% nucleotide sequence identity between the 5' promoter regions of EDN and ECP, we were not surprised to find similar levels of luciferase activity when comparing the different size fragments. Evaluation of the 1.4 kb EDN promoter fragment with the genomatix database and subsequent mutational analysis revealed functional GATA-1 and GATA-2 consensus binding sites in the region between -500 and -1140 in the EDN promoter. In contrast, although consensus GATA-1 and GATA-2 binding sites were present in the ECP 5' promoter, analogous to those of EDN, mutational analysis indicated that these sites had no impact on transcription of ECP. EMSAs demonstrated specific binding of GATA-1 and GATA-2 to the identified consensus elements in the 5' promoter region of EDN. Furthermore, eosinophilic promyelocyte clone 15 cells respond to butyric acid (BA) with increased transcription and translation of GATA-1 and GATA-2 as well as EDN

Conclusion: These results support the proposal that both GATA-1 and GATA-2 binding sites coordinately contribute to transcription of EDN, with each deletion resulting in a decrease in promoter function.

This project was funded by NIAID Division of Intramural Research.

POSTER DISCUSSION SESSION I

POSTER #5

HUMAN EOSINOPHILS EXPRESS FUNCTIONAL NOTCH LIGANDS

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Background: Notch signaling is an evolutionarily conserved pathway dictating crucial cell fate determinations throughout development. More recently recognized are numerous effects of Notch signaling on mature cell functions, including T cell differentiation into Type I, Type II or regulatory phenotypes, B cell activation, tumorigenesis and fibrotic responses. Notch signaling is mediated by interactions between Notch ligand-expressing effector cells and Notch receptor-expressing target cells. Five Notch ligands (Jagged 1 & 2, Delta-like 1, 3 & 4), and four Notch receptors (Notch 1, 2, 3 & 4) have been identified in humans. Despite the increasing recognition of key effects of Notch signaling on mature cell functions and in diseases, the expression and function of Notch ligands by innate immune leukocytes, including eosinophils, are undefined.

Objectives: We investigated the expression and function of Notch ligands by human blood eosinophils.

Methods: Human eosinophils were isolated by negative selection from the blood of healthy and atopic donors and assessed for Notch ligand mRNA and protein expression using real time RT-PCR, western blotting, immunofluorescence microscopy and flow cytometry. Modulation of eosinophil Notch ligand mRNA and protein expression was investigated in response to eosinophil stimulation and fibroblast co-culture. Autocrine functions of Notch signaling for eosinophils were evaluated using 1) real time RT-PCR to detect induction of a Notch-responsive gene in the presence or absence of a Notch signaling inhibitor, and 2) the GAFS (gated autofluorescence forward scatter) assay to investigate a role for Notch signaling in stimulus-induced eosinophil shape change.

Results: Human eosinophils constitutively express Jagged 1 mRNA and protein, and variably express Jagged 2, Delta-like 1 and Delta-like 4 mRNA. Delta-like 3 expression was not detected in any donor. Protein and mRNA expression of Jagged ligands could be maintained in the presence of GM-CSF or upon co-culture with pulmonary fibroblast cell lines in a contact independent manner. Eosinophil-expressed Notch ligands are functional, as evidenced by their mediation within eosinophils of both induction of an early Notch-responsive gene and autocrine induction of eosinophil shape change.

Conclusions: We demonstrate for the first time that human eosinophils express functional Notch ligands. Further, we demonstrate Notch-mediated, autocrine activation of eosinophils. Moreover, increased eosinophil Notch ligand expression upon exposure to pulmonary fibroblast cell lines suggest a means by which these ligands may be modulated on eosinophils in the lungs. In light of recognized functions of Notch signaling in multiple settings from immunomodulation to fibrosis, eosinophil expression of Notch ligands may help to explain observed functional contributions of eosinophils to processes including T cell modulation, pulmonary fibrosis and airway remodeling.

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POSTER DISCUSSION SESSION I

POSTER #6

VESICULAR TRANSPORT OF MAJOR BASIC PROTEIN (MBP) WITHIN ACTIVATED HUMAN EOSINOPHILS

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Background: Classical roles of eosinophils are based on their effector responses involving secretory processes to mobilize and release major basic protein (MBP), one of the eosinophil's four distinctive cationic proteins, implicated in cytotoxicity and mediation of allergic disorders such as asthma. MBP is stored within a dominant population of cytoplasmic granules, termed specific or crystalline granules which exhibit an ultrastructurally unique morphology. The mechanisms of MBP secretion involve granule fusion with the plasma membrane, but little is known about the ability of eosinophils to release MBP through piecemeal degranulation (PMD). This process is based on vesicular transport from the specific granules to the cell surface.

Objectives: We investigated vesicular secretion of MBP within eotaxin-stimulated human eosinophils.

Methods: Eosinophils were isolated from the blood of healthy donors by negative selection and stimulated for 1h with eotaxin (100 ng/mL), a chemokine that induces PMD in eosinophils. Immunolabeling for MBP was performed by immunofluorescence microscopy using agarose preparations and pre-embedding immunonanogold electron microscopy (EM) for precise epitope preservation and subcellular localization. Co-localization of MBP and CD63, a marker of eosinophil granule and vesicle membranes, was also investigated by immunofluorescence. In parallel, vesicle-enriched fractions were isolated from unstimulated eosinophils by subcellular fractionation and prepared for immunonanogold ultrastructural labeling of MBP.

Results: Eosinophils exhibited fluorescent immunoreactive staining for both MBP and CD63 on specific granules. Deconvolution microscopy revealed marginal CD63 granule labeling, often associated with MBP labeling peripheral to CD63, suggestive of extragranular localization. The use of immunonanogold enabled us to detect a prominent system of MBP-containing vesicles around mobilized granules in stimulated eosinophils. Notably, MBP was localized within disarranged cores, matrices and within vesicles extending from or attached to the surface of emptying granules. MBP-containing vesicles included both spherical small vesicles and large tubular carriers (Eosinophil Sombrero Vesicles– EoSVs). Unstimulated eosinophils exhibited a pool of MBP-positive vesicles in the cytoplasm and around granules. In accord, isolated vesicles were positive for MBP.

Conclusion: We demonstrate, for the first time, vesicular trafficking of MBP within human activated eosinophils. This finding identifies PMD as an alternative secretory process to release MBP from eosinophils. Moreover, our results highlight PMD as a distinct and more common degranulation mechanism during eosinophil responses to allergic and inflammatory responses.

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POSTER DISCUSSION SESSION I

POSTER #7

MOLECULAR BIO-MARKERS OF EOSINOPHIL-LINEAGE COMMITMENT: MULTIPLEX Q-PCR ANALYSIS OF GATA-1, MBP AND IL-5 RECEPTOR mRNA EXPRESSION KINETICS.

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Background: Using colony assays and flow cytometry, we have shown that eosinophil/basophil (Eo/B) progenitor phenotype and function are associated with both atopic risk at birth and early childhood clinical outcomes. These assays are cumbersome, however, and we thus endeavoured to develop surrogate molecular markers of eosinophil lineage commitment.

Objectives: To utilize Q-PCR to determine the kinetic patterns of expression of Eo/B-lineage specific genes in cord blood (CB) and peripheral blood (PB) in response to IL-5 stimulation.

Methods: CB and PB non-adherent mononuclear cells (NAMNC) were isolated from random fresh (and frozen, for CB) samples, and incubated in the presence of IL-5 (1 ng/mL). At 24, 48, 72h, 96h (CB only) and 1 week post-stimulation (PB only), RNA was isolated, reverse transcribed, and expression of IL-5Rα, GATA-1, and MBP was determined utilizing multiplex Q-PCR. Relative expression ratios of stimulated to un-stimulated cells were calculated using the delta-delta Ct method.

Results: Stimulation of CB NANMC with IL-5 resulted in an up-regulation of GATA-1 expression, peaking at 48h, and decreasing expression by 72 hours. Preliminary evaluation of a 96h time-point suggests down-regulation. PB NAMNC's similarly showed up-regulation peaking at 48h but with a lower overall fold-increase and a slower return to baseline expression than that observed in CB. MBP expression in CB was slowly up-regulated in response to IL-5 stimulation, maximal at 96h; in PB, MBP expression was stable until after a full week of incubation when up-regulation could finally be detected. There was completely stable expression IL-5Rα, in both CB and PB.

Conclusion: Multiplex Q-PCR analysis of mRNA from CB and PB demonstrates expression of critical Eo/B lineage-specific events. Further investigation of the validity and utility of Q-PCR analyses of CB and PB for surrogate, molecular markers of Eo/B differentiation is underway.

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POSTER DISCUSSION SESSION I

POSTER #8

REGULATION OF MMP-9 RELEASE BY HUMAN EOSINOPHIL MIGRATION

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Background: To initiate eosinophil migration *in vitro,* 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE), a potent eosinophil chemotactic factor, activates proteolysis, notably by promoting matrix metalloproteinase (MMP)-9 secretion.

Objectives: We postulated that protein kinase C (PKC) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) are involved in 5-oxo-ETE-induced eosinophil migration through extracellular matrix by increasing MMP-9 secretion.

Methods: Purified peripheral blood eosinophils were pre-incubated with or without different selective PKC inhibitors (isoforms α β: Ro-31-8425, isoform δ: Rottlerin or PKC ζ blocking peptide), ERK inhibitor (PD98059) or p38 MAPK inhibitor (SB203580) for 30 min at 37°C, followed by addition of 5-oxo-ETE. Migration assays using Matrigel, a reconstituted basement membrane, was assessed, and rapid MMP-9 release (within an hour) was evaluated by zymography.

Results and Conclusion: All of these inhibitors decreased by 30 to 90% 5-oxo-ETE-induced eosinophil migration through Matrigel. However, only ERK signalling pathway inhibitor reduced rapid release of MMP-9, whereas both PKC δ and ERK are implicated in MMP-9 synthesis suggesting that the other kinases studied could act on MMP-9 synthesis, generation of other proteases, adherence or cell motility. More experiments are needed to clarify the mechanisms that regulate eosinophil migration.

Funded by the Canadian Institutes of Health Research.

POSTER DISCUSSION SESSION I

POSTER #9

TEMPORAL DIFFERENCES IN CORTICOSTEROID EFFECTS ON OPSONIN AND ADHESION RECEPTORS ON HUMAN EOSINOPHILS

Willem ten Hove[1,2], Leo A. Houben[2], René C. Schweizer[2], Hubertus G.M. Leufkens[1], Jan A.M. Raaijmakers[1], Leo Koenderman[2] and Madelon Bracke[1,2]

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Background: Corticosteroids inhibit inflammation at many levels: reduction of cytokine production and inhibition of survival, differentiation, recruitment and activation of inflammatory cells. Most of the corticosteroid actions occur after prolonged incubation and act by genomic processes. In vivo, also rapid (non-genomic) effects of steroids have been described, however, the mechanisms of direct effects of corticosteroids on inflammatory cells are poorly defined.

Objectives: To study the effects of corticosteroid treatment on eosinophil functional responses, in vitro.

Methods: The effects of corticosteroids were investigated on freshly isolated eosinophils from blood of healthy donors. Both unprimed and IL-5 primed eosinophils were treated either short-term (<30 min) or overnight (16 hrs), with dexamethasone (10^{-6}-10^{-12}M). Functional assays (adhesion, migration, Fc-receptor activation) were performed to analyze the effects of corticosteroids on eosinophil priming and activation.

Results: In this study we show a rapid, most likely non-genomic, priming effect of corticosteroids on eosinophil effector functions. This effect appears to be highly specific, affecting only FcαRI (CD89), while FcγRII and cell adhesion receptors (integrins) are not affected. Short-term incubation with dexamethasone resulted in a rapid increase in binding of IgA-beads, but not IgG-beads, to human eosinophils (n=8 experiments; $p<0.005$). This priming response by dexamethasone was dose dependent and optimal between 10^{-8}M to 10^{-6}M (n=3; $p<0.005$). In contrast, no rapid effects on β_2-integrin (CD11b/CD18) expression or activation (binding of ICAM-1 beads; n=3) could be detected. However, when treated with dexamethasone overnight (16 hrs), adhesion and migratory responses of IL-5 primed eosinophils could be affected. We found that IL-5 induced chemokinesis of eosinophils was significantly inhibited by overnight incubation with dexamethasone with an optimal concentration of 10^{-6}M (n=3; $p<0.05$). Overnight treatment with dexamethasone also inhibited IL-5 induced $\alpha_m\beta_2$-integrin activity, as measured by a decreased binding of ICAM-1 beads (n=3; $p<0.05$).

Conclusions: This work demonstrates that 1) short-term treatment with corticosteroids selectively primes the functionality of the FcαRI on eosinophils, without affecting adhesion and migration, and 2) overnight corticosteroid treatment inhibits migratory and adhesive responses, specifically in primed eosinophils. These data underscore a tight regulation of eosinophil functionality and the effects of steroids.

POSTER DISCUSSION SESSION I

POSTER #10

EOSINOPHIL FUNCTIONS INDUCED BY ADHESION MOLECULES AND LEUKOTRIENE D_4

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Background: Eosinophils preferentially accumulate at sites of airway inflammation of asthma. For circulating eosinophils to participate in the asthmatic airways, it is necessary to interact with adhesion molecules expressed on endothelial cells and then expose to inflammatory mediators such as cysteinyl leukotrienes(cysLTs). There is evidence that cysLTs including leukotriene (LT)D_4 regulate the functional status of eosinophils.

Objectives: To investigate whether interaction with adhesion molecules modifie eosinophil functions induced by cysLTs.

Methods: rh-VCAM-1, rh-ICAM-1, or rh-P-selectin was dissolved in 0.05 M $NaHCO_3$ coating buffer, added to 96-well EIA plates and incubated at 4 ^oC overnight . Residual fluid was decanted and HBSS/0.1% gelatin was added to reduce non-specific activation of eosinophils. Eosinophils were isolated from blood of healthy donors, incubated in the EIA plates, and then exposed to LTD_4. The generation of superoxide anion (O_2^-) and release of eosinophil-derived neutrotoxin (EDN) were evaluated by cytochrome C reduction assay and ELISA, respectively.

Results: In this experimental condition, neither VCAM-1 nor LTD_4 (100nM) directly induced eosinophil O_2^- generation, however, VCAM-1 and LTD_4 act synergistically to induce eosinophil O_2^- generation. The O_2^- generation induced by a combination of VCAM-1 and LTD_4 was blocked by anti-α_4 integrin mAb and anti-β_2 integrin mAb. ICAM-1 by iteslf induced eosinophil O_2^- generation and this was enhanced by LTD_4. The enhanced O_2^- generation was blocked by anti-β_2 integrin, but not anti-α_4 integrin mAb. P-selectin did not induce O_2^- generation in the presence or absence of LTD_4. Finally, a combination of LTD_4 and VCAM-1, but not ICAM-1 or P-selectin, induced the release of EDN.

Conclusions: The combination of VCAM-Ior ICAM-I and cysLT effectively induce effector functions of eosinophils. Eosinophil adhesion to and migrate across endothelial cells via these speficic adhesion proteins and subsequent exposure to cysLT may be involved in the manifestations of eosinophil activation in the airways of asthma.

POSTER DISCUSSION SESSION I

POSTER #11

THE EFFECT OF CATIONIC CHARGE ON RELEASE OF EOSINOPHiL MEDIATORS: ROLE OF SURFACE RECEPTORS

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Background: In patients with atopic diseases, cationic charged eosinophil proteins are present in inflamed tissues. While the role of cytokines in cell activation is well established, the presence of tissue cationic charged material may also be an important factor in inflammatory cell function.

Objectives: To determine if increased cationic charge seen in an atopic microenvironment plays a role in the activation of eosinophils.

Methods: Human eosinophils were incubated with sepharose beads coated with cationic or anionic compounds in the presence and absence of a cytokine cocktail (IL-3, IL-5, GM-CSF) to simulate the milieu of inflammation. Eosinophil peroxidase (EPO) and eosinophil derived neurotoxin (EDN) release were compared to eosinophil morphology and expression of CD18 and CD89, determined by confocal microscopy.

Results: Cytokines with positively charged beads caused greater EPO release (lysine-coated, 44.2 nM, compound 48/80, 40.0nM, or EDN-coated, 49.1 nM) than cytokines alone (14.9 nM). Beads coated with heparin, dextran sulfate, and aspartic acid blocked this effect. EDN release was also induced by lysine-coated beads with cytokines (67.1 ng/100μl) and blocked by heparin. Eosinophil incubation with wortmannin, genistein, and the src kinase inhibitor, PP1, blocked cationic signaling. Inhibition of CD18 but not eosinophil adhesion prevented the cationic charge effect. Cationic charge induced increased expression of CD18 and CD89 at the bead surface, which was prevented by anionic charge coating.

Conclusion: Cationic charged surfaces induce eosinophil mediator release by polarizing expression of cell surface receptors.

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POSTER DISCUSSION SESSION I

POSTER #12

HOUSE DUST MITE ALLERGEN ACTIVATES HUMAN EOSINOPHILS VIA FORMYL PEPTIDE RECEPTOR AND FORMYL PEPTIDE RECEPTOR-LIKE 1

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Background: Inhalant allergens can directly activate human eosinophils in the absence of other immune cells.

Objectives: To determine which receptors and signaling pathways the aeroallergens house dust mite (HDM) and birch pollen engage to activate human eosinophils.

Methods: Chemotaxis and degranulation evoked by the allergens was studied in eosinophils pre-treated with pertussis toxin and other antagonists of G protein-coupled receptors, e.g. the formyl peptide receptor (FPR), CC chemokine receptor 3 (CCR3) and leukotriene receptor B4 (LTB4R). Pharmacologic inhibition of signal transduction molecules was also assayed. Receptor-transfected HL-60 cells and neutrophils were examined for comparative purposes.

Results: Inhibition of the FPR, as well as desensitization of the receptor rendered eosinophils anergic to activation by the allergens, measured as chemotaxis and EPO release. Blockade of CCR3 or LTB4R did not affect eosinophilic reactivity. It was determined by PCR that human eosinophils express the formyl peptide receptor family members FPR and FPR-like 1 (FPRL1). HDM, unlike birch pollen, evoked calcium fluxes in HL-60 cells transfected with FPR or FPRL1. Although both allergens gave rise to calcium transients in neutrophils, which also express FPR and FPRL1, only the HDM response was decreased by the FPR antagonist. Moreover, neutrophils migrated toward HDM but not to birch pollen. Eosinophils pre-treated with inhibitors of MAPK p38, ERK1/2 and protein kinase C exhibited diminished responsiveness to the aeroallergens.

Conclusions: This study indicates that FPR and FPRL1 mediate the activation of eosinophils by HDM, whereas birch pollen employs other pathways shared with FPR to activate human eosinophils.

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POSTER DISCUSSION SESSION I

POSTER #13

RAB27A IN HUMAN EOSINOPHILS: DIFFERENTIAL ACTIVATION PATTERNS

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Background: Eosinophils play an effector role in airway damage in asthma and related disorders, since their secreted granule-stored products can induce many of the clinical features of asthma. Degranulation of eosinophils requires the activity of several GTP-binding proteins, many of which remain unidentified. We hypothesized that Rab27A is one of these GTP-binding proteins because, in other cells, it interacts with effectors that regulate vesicle motility, docking and fusion. Subjects with Rab27A-deficiency develop Griscelli syndrome which is characterized by immunodeficiency and partial albinism due to secretory defects in various cell types.

Objectives: To assess Rab27A activation in human eosinophil degranulation.

Methods: RT-PCR and Western blotting were performed on human peripheral blood eosinophils. To asses Rab27A activation, we developed a novel assay allowing the pulldown of the active, GTP-bound form.

Results: Rab27A was expressed in human peripheral blood eosinophils and transiently activated by secretory-IgA stimulation. The kinetics of Rab27A activation varied between donors. High eosinophilic donors (>$25x10^6$cells/100ml of blood) displayed a more rapid cycle of Rab27A activation and inactivation as compared to eosinophils from other donors (<$15x10^6$cells/100 ml of blood). Rab27A was also expressed in the HL-60 clone 15 'eosinophil-like' cell line, but was not expressed in AML14.3D10 cells. In the HL-60 clone 15 cells, Rab27A was also activated by sIgA stimulation with activation pattern similar to that of peripheral blood eosinophils although the kinetics were much slower. Rab27A was also activated by IFN-γ and eotaxin in these cells.

Conclusions: Rab27A is activated in human eosinophils following sIgA stimulation suggesting it may induce secretory granule movement and exocytosis in these cells. The differential activation pattern of Rab27A provides a pattern of the differences between resting and activated eosinophils.

Funding provided by the Canadian Institutes of Health Research and the Alberta Heritage Foundation for Medical Research

POSTER DISCUSSION SESSION I

POSTER #14

DIRECT *MYCOBACTERIUM BOVIS*-BCG RECOGNITION BY HUMAN EOSINOPHILS INVOLVES TOLL-LIKE RECEPTOR 2 AND TRIGGERS α-DEFENSIN PRODUCTION.

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Background: Eosinophils are multifunctional leukocytes involved in various inflammatory processes. Recent studies provide evidence that eosinophils can exert different functions through their ability to express different receptors and to produce both Th1- and Th2-type cytokines upon stimulation. Eosinophils play potent immunoregulatory functions during cancer, inflammation, auto-immune diseases and infections. However, their exact function in innate immune responses remains unclear. By contrast with extensive studies of eosinophil function in helminth infections, little is known about the role of eosinophils in host defense against other pathogens such as viruses, fungi and bacteria.

Objectives: The role of eosinophils in mycobacterial pathogenesis is still not well defined. Recently, an accumulation of eosinophils within lung granuloma was reported in different models of mycobacterial infections, while a lytic activity against *M. tuberculosis* was assigned to eosinophil peroxydase (EPO). The aims of this study were to demonstrate a direct interaction of human eosinophils with mycobacteria and to analyse the mechanisms involved.

Methods: TLR expression and functionality was investigated on eosinophils from normal donors and allergic patients using RT-PCR, flow cytometry, immunofluorescence, and chemiluminescence assays.

Results: We demonstrate that *M.bovis*-BCG attracts and activates eosinophils *in vitro*. Indeed, eosinophils produce Reactive Oxygen Species (ROS) and release EPO in the presence of BCG. We report here that only TLR2, but not TLR4 is involved in eosinophil/ BCG interactions through activation of MAP kinase signalling. Finally, we show that eosinophils can exert cytotoxic functions against BCG through production of defensins.

Conclusions: This work represents the first demonstration of a direct interaction between human eosinophils and *M. bovis*-BCG and suggests a novel role for eosinophils in TLR2-mediated innate immunity. Furthermore, defensins act as a novel cytotoxic pathway for eosinophils. Therefore, the role of eosinophils in the host immune response to mycobacterial infections in experimental and clinical tuberculosis remains to be established.

POSTER DISCUSSION SESSION I

POSTER #15

IDENTIFICATION OF INTRACELLULAR LIGANDS FOR HUMAN GALECTIN-10 (CHARCOT-LEYDEN CRYSTAL PROTEIN) IN EOSINOPHILS: NOVEL INTERACTIONS WITH GRANULE CATIONIC RIBONUCLEASES

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Background: The human eosinophil Charcot-Leyden Crystal (CLC) protein, which forms distinctive hexagonal bipyramidal crystals considered hallmarks of eosinophil participation in allergic responses and related inflammatory reactions, comprises ~7-10% of total eosinophil protein and was originally suggested to be a lysophospholipase. However, we have reported that CLC protein is not a lysophospholipase, but is instead a member of the galectin superfamily of animal lectins, and it is now designated galectin-10 (Gal-10). Gal-10 has now been reported to be expressed by CD4+/CD25+/Foxp3+ regulatory T cells and to be required for their functional activity (Kubach et al Blood 2007, in press). The oligosaccharide-containing glycoprotein or proteoglycan ligands of CLC/Gal-10, its cellular function in eosinophil biology, and role in allergic diseases have not been elucidated.

Objectives: The goal of this study is to better understand the function(s) of CLC/Gal-10 in eosinophil biology and its potential role in eosinophil piecemeal degranulation.

Methods: Ligand blotting, co-immunoprecipitation, and confocal microscopy were used to identify intracellular CLC/Gal-10 ligands in eosinophils, interactions of CLC/Gal-10 with the identified proteins, and their co-localization during eosinophil activation, respectively.

Results: We show that CLC/Gal-10 interacts both *in vitro* and intracellularly with two glycosylated human eosinophil granule cationic ribonucleases, eosinophil-derived neurotoxin (EDN, RNS2) and eosinophil cationic protein (ECP, RNS3). CLC/Gal-10 also binds avidly to murine eosinophil-associated ribonucleases, despite the absence of a CLC/Gal-10 ortholog in the mouse. The binding of CLC/Gal-10 to the eosinophil ribonucleases does not require they be glycosylated, since glycosidase-mediated cleavage of N- and O-linked glycans from EDN did not inhibit binding, and CLC/Gal-10 also binds to non-glycosylated EDN expressed in *E. coli*. Studies using purified blood eosinophils and immunofluorescence confocal microscopy show that in resting, non-activated eosinophils, CLC/Gal-10 protein resides in the cytosolic compartment of the cell. In contrast, eosinophil activation with IFN-γ for 10-30 minutes induces rapid co-localization of CLC/Gal-10 with both EDN/RNS2 and the tetraspanin CD63, a marker of eosinophil secondary granules and secretory vesicles, during the process of eosinophil piecemeal degranulation. Finally, CLC/Gal-10 does not inhibit the ribonuclease activity of EDN.

Conclusions: For this reason, we suggest that CLC/Gal-10 functions instead as a carrier for sequestration and vesicular transport of these potent cationic ribonucleases/cellular toxins during eosinophil piecemeal degranulation, enabling their extracellular functions in host defense against parasitic helminths and in allergic inflammation, without intracellular damage to the eosinophil itself during their secretion.

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POSTER DISCUSSION SESSION I

POSTER #16

EOSINOPHIL-DERIVED NEUROTOXIN (EDN) AND EOSINOPHIL CATIONIC PROTEIN (ECP) AS POTENTIAL ENDOGENOUS LIGANDS OF THE TOLL-LIKE RECEPTOR-2 SIGNAL TRANSDUCTION PATHWAY

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Background: Toll-like receptors (TLRs) predominantly utilize the MyD88-dependent, NF-κB signal transduction pathway. Yang *et al* (in press) has shown that recombinant eosinophil derived neurotoxin (rEDN) acts as an endogenous ligand for TLR2 by activating the same NF-κB pathway in otherwise naïve human embryonic kidney (HEK293) cells when transiently co-transfected with an NF-κB reporter luciferase construct and a plasmid expressing TLR2, but not TLR3 or TLR4. Given these data, we postulate that eosinophil cationic protein (ECP), and potentially other related RNase A ribonucleases, may also signal via TLR2. We also postulate that unique structural elements within these ribonucleases are necessary for eliciting this signal. **Objectives:** We intend to determine whether ECP and/or other members of the RNase A gene superfamily also act as endogenous ligands of TLR2. We also plan to investigate whether or not enzymatic activity and/or tertiary structure are necessary for EDN-mediated TLR2 signalling. **Methods:** HEK293 cells were transiently co-transfected with a NF-κB firefly-luciferase reporter construct and pTLR-2 for 24 hours, then stimulated with indicated concentrations of either peptidoglycan (PGN, Sigma), human EDN, ECP (Developmental Diagnostics), Bovine RNase A (Sigma), commercial urinary gonadotropin containing human EDN (Sigma) or purified human RNase 1 (Sigma) for 48 hrs at 37℃. Luciferase activity was measured by using the Dual Luciferase Reporter Assay System (Promega). Renilla luciferase plasmid was used as a control for efficiency of transfection.

Results: Experiments performed by Yang *et al.* indicate that bacterial-derived recombinant EDN promotes signal transduction uniquely via TLR2. Using a similar system, we observe a dose-response to PGN with 8-fold and 15-fold increases in luciferase activity with the addition of 1μg/mL and 5μg/mL, respectively, thus documenting the creation of a functional TLR2-dependent response pathway. However, our initial results reveal that ribonucleolytically-active ECP and EDN purified from normal human eosinophils do not activate the TLR2/MyD88 pathway. Similarly, bovine ribonuclease A does not promote signal transduction via TLR2.

Conclusions: Although bacterial-derived recombinant EDN signals via the TLR2/MyD88 pathway, EDN and ECP purified from normal human eosinophils, as well as bovine RNase A, do not appear to do likewise. This suggests that the source and preparation of the ribonucleases may have an impact on their ability to generate signal through TLR2. Further studies are currently underway to elucidate if human RNase 1 and EDN purified from commercial urinary gonadotropin preparations might promote signal transduction via TLR2.

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POSTER DISCUSSION SESSION I

POSTER #17

A HIGH RF LEVEL INDICATES EOSINOPHIL ACTIVATION, AND IS A CLINICAL MARKER OF SEVERITY OF ASTHMA.

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Background: It has been reported that certain patients with asthma are rheumatoid factor (RF)-positive. However, it has not been established whether RF levels are clinical markers of asthma.

Objectives: In this study we investigated the relationship between RF, severity of asthma and eosinophil activation.

Methods: Levels of RF and numbers of peripheral eosinophils were assessed during asymptomatic periods in 54 patients with mild asthma and 38 patients with moderate asthma treated without systemic steroids. The same parameters were also assessed in 14 of these patients during asthma attacks. In a different group of 31 asthmatic patients, we measured RF levels and surface expression of CD11b on eosinophils.

Results: RF levels and eosinophil counts were significantly higher in moderate than in mild asthma ($P<0.05$). The number of peripheral eosinophils and RF level during attacks were significantly higher than during asymptomatic periods ($P<0.05$). The density of CD11b expression on eosinophils was significantly greater in the patients with high RF levels ($\geq$21 IU/ml) than in those with low RF levels ($P<0.05$).

Conclusions: High RF levels are associated with both asthma severity and eosinophil activation.

POSTER DISCUSSION SESSION I

POSTER #18

PEPTIDE-NUCLEIC ACID-MEDIATED KNOCKDOWN OF THE IL-5-DEPENDENT GENE, PIM-1, IN HUMAN PERIPHERAL BLOOD EOSINOPHILS LEADS TO A DECREASE IN IL-5-MEDIATED CELL SURVIVAL

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Background: The presence of activated eosinophils (eos) in the lungs of asthmatic patients has been proposed to contribute to the airway remodeling and fibrosis that is associated with asthma. It has been demonstrated that allergic asthmatics exhibit elevated systemic and tissue levels of the cytokine interleukin-5 (IL-5), which is thought to play a role in the increased numbers of eos found in the blood and airway. This accumulation of eos, coupled with the ability of IL-5 family cytokines to promote cell survival, contributes to the accumulation of these cells in the affected tissues. However, the underlying intracellular mechanisms whereby IL-5 family cytokines increase eos survival are not clearly understood. In this regard, Pim-1 is an anti-apoptotic kinase that has been reported to mediate cytokine-dependent survival in some cell types. Accordingly, we hypothesize that IL-5-dependent eos survival is mediated, at least in part, through the expression of the serine/threonine kinase, Pim-1.

Objectives: To examine the role of the anti-apoptotic kinase Pim-1 in IL-5-mediated eos survival using a novel strategy to manipulate eos gene expression (i.e. peptide nucleic acid (PNA) antisense directed against Pim-1).

Methods: Human eos were incubated with 100 pM IL-5 for varying times, cell lysates were prepared, and Pim-1 protein expression was assessed via immmunoblotting. Blood eos were incubated with control buffer, protein transduction reagent (Pep-2/Chariot II) alone, targeted PNA alone, or varying concentrations of targeted PNA complexed with Pep-2 for 1 hr. The cells were then suspended in a final volume of media and stimulated with 100 pM IL-5 for 24 hr, after which cell lysates were generated and Pim-1 protein expression was assessed via immunoblotting. Viability of the cells was determined using propidium iodide staining at 48 hr.

Results: Stimulation of peripheral blood eos results in increased expression of Pim-1 protein as early as 45 min after treatment and this expression persists out to 24 hr. Incubation with targeted PNA/Pep-2 complexes results in inhibition of IL-5-dependent Pim-1 expression in a dose-dependent manner (50-90% reduction in protein expression, n=5). Incubation with 1.2 μM targeted PNA/Pep-2 complexes followed by 48 hr stimulation with 100 pM IL-5 results in a decrease in IL-5-mediated eos survival, as assessed by PI staining (4 fold increase in PI staining compared to IL-5 alone, n=3).

Conclusions: These data demonstrate that targeted PNA inhibition of protein expression is highly effective at attenuating the expression of the IL-5-induced gene Pim-1 in primary blood eos. Furthermore, the present data suggest a key role for Pim-1 in IL-5-mediated eos survival. These data also illustrate that PNA-mediated knockdown of IL-5-dependent gene expression is a novel technique that will should useful in dissecting multiple signaling pathways that are operative in primary human eos.

POSTER DISCUSSION SESSION I

POSTER #19

RETINOIC ACIDS INDUCE SURVIVAL AND CYTOKINE PRODUCTION IN EOSINOPHILS

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Background: Retinoic acids (RAs), all-trans RA (ATRA) and 9-cis RA are active metabolites of vitamin A and known to regulate cell proliferation, differentiation, and apoptosis. These effects are mediated by ligand-dependent activation of the nuclear RA receptors (RARs) and retinoid X receptors (RXRs). Although RAs are critical modulator of granulocyte differentiation, their function on eosinophil is unclear so far.

Objectives: In the present study, we sought to examine the expression of RA receptors on eosinophils and the effects of RAs on eosinophil survival and cytokine/chemokine production**.**

Methods: The expression of RA receptors on purified human peripheral blood eosinophils was examined by RT-PCR and Western blotting. Eosinophils were cultured with ATRA or 9-cis RA and then stained with Annexin V and PI, and cell survival was determined using flow cytometer. Production of cytokines in cell culture supernatants was measured by cytokine antibody array and ELISA.

Results: Several subtypes of RARs and RXRs were expressed by eosinophil. ATRA and 9-cis RA significantly prolonged eosinophil survival in a concentration-dependent manner. The neutralizing anti-IL-5 or anti-GM-CSF antibodies failed to inhibit the effect of RAs. The array data and ELISA showed increased production of MCP-1 by eosinophil stimulated with RAs.

Conclusions: RAs may regulate eosinophil survival, which is independent of autocrine production of IL-5 and GM-CSF. RAs also activate eosinophil to produce MCP-1. These findings indicate a novel role for RAs on eosinophil function and provide further insight into pathogenesis of eosinophil-related diseases.

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POSTER DISCUSSION SESSION I

POSTER #20

PROCATEROL ENHANCES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)Γ EXPRESSION IN HUMAN EOSINOPHILS THROUGH BETA2-ADRENOCEPTOR

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Background: Peroxisome proliferator-activated receptor (PPAR) γ is a nuclear receptor that regulates immune reaction. We have previously demonstrated that human eosinophils express PPARγ and that stimulation with a synthetic agonist for PPARγ attenuated the factor induced eosinophil activations. However, the modulator of PPARγ expression in eosinophils has not yet been studied.

Objectives: In this study, we investigated the effect of procaterol, the synthetic beta2-adrenoceptor agonist widely used as bronchodilators in asthma, on the PPARγ expression in eosinophils.

Methods: Purified human peripheral blood eosinophil and the eosinophilic cell line EoL-1 were cultured with procaterol. This was followed by PPARγ measurement by using flow cytometer and quantitative real-time RT-PCR.

Results: We observed that PPARγ was constitutively expressed by EoL-1 and the purified eosinophils, and that the therapeutic concentration (10^{-9}M) of procaterol markedly enhanced PPARγ protein and mRNA expression in EoL-1 and eosinophils, which was reversed by the selective beta2-adrenoceptor antagonist ICI-118551.

Conclusions: These findings suggest that procaterol could modulate the eosinophil function by increasing the expression of PPARγ.

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POSTER DISCUSSION SESSION I

POSTER #21

PGD_2 ELICITS DIFFERENTIAL RELEASE OF PRE-FORMED CYTOKINES FROM EOSINOPHILS: COOPERATIVE SIGNALING BETWEEN DP AND CRTH2 RECEPTORS

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Background: Eosinophils store a multitude of pre-formed cytokines with diverse biologic activities. Relevant to potential immunological roles, eosinophils have distinctive secretory mechanisms by which they rapidly select specific stored cytokines to be released by means of vesicular transport. We hypothesized that the differential release of pre-formed cytokines from eosinophils is a highly regulated and stimulus-specific process. Among potential inducers of such refined process, PGD_2 appear as an interesting candidate. PGD_2, a well-established eosinophilotactic factor, was recently recognized as also capable of triggering eosinophil activation, including lipid body biogenesis and LTC_4 synthesis.

Objectives: Here, we investigated whether prostaglandin (PG)D_2 is able to activate cytokine secretory machinery of eosinophils.

Methods: The profile of cytokine content of cell-free supernatants and whole cell lysates was obtained by multiplexed analysis according to manufacturer's instructions (BioRad).

Results: Analysis of 17 human cytokines/chemokines (IL-1β/IL-2/IL-4/IL-5/IL-6/IL-7/IL-8/IL-10/IL-12p70/IL-13/IL-17/G-CSF/GM-CSF/IFN-γ/TNF-α/MCP-1/MIP-1β) within whole cell lysates of purified human eosinophils revealed that all cytokines analyzed are stored as pre-formed pool. *In vitro* stimulation with PGD_2 (25nM) was able to rapidly (1h) induce specific release of stored IL-2, -4, -6, G-CSF, GM-CSF, IFN-γ, TNF-α, MCP-1 and MIP-1β. Selective activation of either type of PGD_2 receptor by selective agonists such as BW245c (DP receptor) or DK-PGD_2 (CRTH2 receptor) induced secretion of complementary selection of cytokines, respectively, IL-2, -6, G-CSF, GM-CSF, TNF-α, MCP-1 and MIP-1β *versus* IL-4, -6, GM-CSF, IFN-γ and TNF-α. *In vivo*, using a mouse model of allergic inflammation, we verified that infiltrating eosinophils are embedded in an inflammatory fluid packed with cytokines, including IL-6, -10, TNF-α, MCP-1, KC and RANTES, detected by simultaneous analysis of 10 mouse cytokines (IL-1β/IL-4/IL-6/IL-10/IL-12p70/IFN-γ/TNF-α/MCP-1/KC/RANTES). Pre-treatment with HQL-79 (PGD-synthase inhibitor) inhibited allergen-triggered release of cytokines (but TNF-α), indicating that PGD_2 is an endogenous regulator of cytokine release during eosinophilic inflammation.

Conclusions: Both PGD_2 receptors expressed on eosinophils cooperatively signal to select the specific array of cytokines secreted in response to PGD_2, which in turn may endow eosinophils with regulatory roles in acquired immunity.

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POSTER DISCUSSION SESSION I

POSTER #22

MONTELUKAST REGULATION OF CYSTEINYL LEUKOTRIENE RELEASE BY BLOOD EOSINOPHILS

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Background: Cysteinyl leukotrienes (CysLT) (LTC4, LTD4 and LTE4) play major roles in asthma pathogenesis. Asthma is characterized by leukocyte infiltration in the bronchial mucosa, notably eosinophils (Eo), which are a main source of CysLT. Montelukast (MTK), a CysLT receptor antagonist used in the treatment of asthma, diminishes the inflammation caused by CysLT.

Objective: In this study, we evaluated the effect of MTK on the calcium ionophore (A23187)-induced LTC4 release in isolated Eo.

Methods: Asthmatics' blood Eo (n = 4) were incubated with either MTK, the 5-lipoxygenase-activating-protein antagonist MK-0591, or Pertussis toxin (PTX), which inactivates Gαi-proteins, putatively coupled to CysLT receptor. Eo were then stimulated with A23187 (100 nM). LTC4 levels were determined in cell-free supernatants by enzyme-linked immunoassay.

Results: MTK decreased LTC4 release by 10 to 80% in a dose-dependent manner. This effect was significant at 1 and 10 µM ($p<0.001$). MK-0591 (10 nM), used as positive control, decreased LTC4 release by 92% ($p=0.01$). PTX (25 ng/ml) decreased the LTC4 release by 30% ($p=0.03$); such an effect was not sufficient to support an inhibition of LTC4 autocrine stimulation by MTK.

Conclusions: These data suggest that MTK decreases LTC4 release by a mechanism unrelated to CysLT receptor blockade. The molecular target involved in this inhibitory effect of MTK on LTC4 release remains to be unraveled.

POSTER DISCUSSION SESSION I

POSTER #23

RESVERATROL INHIBITS EOSINOPHIL PEROXIDASE-INDUCED MAST CELL DEGRANULATION

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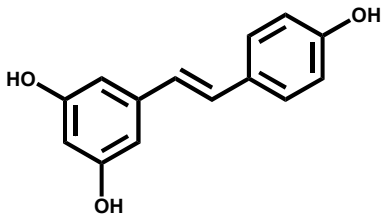
Background: Eosinophil peroxidase (EPO) is a major enzyme present in eosinophils, and it is released into asthmatic airways upon eosinophil degranulation. EPO, when combined with its substrates, hydrogen peroxide and a halide, has been shown to cause histamine release from human mast cells. Resveratrol, a natural polyphenolic compound found in the skin of red grapes, has been shown to have anti-inflammatory, anti-oxidant, cardio-protective and anti-tumor properties. It has been reported that resveratrol can act as an irreversible mechanism-based inactivator of enzymatic peroxidation, inhibiting, for example the peroxidation reactions catalysed by cyclooxygenase-1 and myeloperoxidase.



Resveratrol

Objectives: The aims of this research were to evaluate the potency of resveratrol as an inhibitor of EPO catalysis and to determine resveratrol's ability to dose-dependently inhibit EPO-induced mast-cell histamine release.

Methods: Two independent enzyme assays were used to measure human EPO catalytic activity. In addition, mouse peritoneal mast cells were treated with a combination of EPO and the substrates, hydrogen peroxide and bromide, resulting in mast cell degranulation, the extent of which was determined by quantifying histamine release.

Results: EPO was found to be a highly effective instigator of mast cell degranulation, at physiologically relevant levels of enzyme, but only in conjunction with both substrates. Neither EPO nor hydrogen peroxide alone was found to effect histamine release to any significant extent. Resveratrol was found to be a potent EPO inhibitor and an effective modulator of EPO-induced mast cell degranulation.

Conclusions: These results suggest a novel mechanism of action for the naturally occurring anti-oxidant in mediating its reported anti-inflammatory properties.

POSTER DISCUSSION SESSION I

POSTER #24

TRYPTOPHAN CATABOLITES MODULATE EOSINOPHIL DEGRANULATION THROUGH GLUTAMATE RECEPTORS

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Background: Glutamate is an excitatory neurotransmitter in the central nervous system. Prolonged exposure to glutamate leads to neuronal apoptosis through a process called excitotoxicity. Quinolinic acid and Kynurenic acid, products of oxidative catabolism of tryptophan are agonists and antagonists on NMDA glutamate receptors, respectively. The expression of glutamate receptors in extra-neuronal cells may be important in cellular activation and apoptosis. We hypothesize that the differential expression of glutamate receptors on inflammatory and immune cells may play a role in the antiproliferative and pro-apoptotic effects of tryptophan catabolites on lymphocytes but not eosinophils.

Objectives: The main objectives of this study are to determine the expression of glutamate receptors and transporters on human eosinophils, and the effect of glutamate receptor activation on eosinophil degranulation.

Methods: Reverse-transcription/Polymerase Chain Reaction (RT-PCR) and flow cytometry were used to determine the expression of ionotropic (NMDA, AMPA and Kainate) and metabotropic (mGluR1-mGluR8) glutamate receptors. The expression of transporters and the modulation of their expression by TLR agonists were determined using RT-PCR. Flow cytometry was used to estimate changes in intracellular calcium in eosinophils loaded with calcium-sensitive dyes, FURA-2 and –3. Intracellular cAMP was measured colorimetrically. Following treatment with glutamate receptor agonists, the release of eosinophil peroxidase (EPO) was measured colorimetrically using OPD.

Results: Human eosinophils expressed both metabotropic (mGluR2 and mGluR7) and ionotropic (NMDA) glutamate receptors. Freshly isolated human eosinophils did not express any of the six currently known classes of glutamate transporters. However, treatment of eosinophils with GM-CSF or agonists of TLR-3 (Poly I-C), TLR-7 (loxoribine), and TLR-9 (CpG) rapidly induced the expression of the Xc^- cystine/glutamate antiporter system. Treatment of eosinophils with glutamate, NMDA or Quinolinic acid increased intracellular calcium concentration in a glutamate-free medium. Similarly, NMDA and Quinolinic acid directly induced the release of EPO in human eosinophils. The effect of NMDA was inhibited by MK-801, a competitive antagonist of NMDA.

Conclusions: Eosinophils express functional glutamate receptors that can be activated by tryptophan catabolites. Unlike lymphocytes, which express mGluR1, mGluR3, and mGluR5, human eosinophils express mGluR2 and mGluR7. The expression of cystine/glutamate antiporter system is linked to activation of TLR receptors, suggesting that intracellular glutamate transactions may play a role in innate immunity.

Funding: AHFMR, CIHR, AllerGen.

POSTER DISCUSSION SESSION I

POSTER #25

A SELDI-TOF MS STUDY OF THE MOLECULAR HETEROGENEITY OF EOSINOPHIL CATIONIC PROTEIN.

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Background: Eosinophil cationic protein (ECP) is a highly heterogeneous protein due to gene polymorphisms and post-translational modifications of the protein.

Objective: To further investigate ECP heterogeneity. Hence, an affinity capture assay based on an antigen-antibody interaction with the Surface Enhanced Laser Desorption/Ionization- Time of Flight Mass Spectrometry (SELDI-TOF MS) technique was developed.

Methods: MS analysis of ECP was performed by SELDI-TOF MS, using monoclonal anti-ECP antibodies coupled to PS20 ProteinChip arrays. ECP heterogeneity of single individuals was studied in extracts of purified blood eosinophils. ECP purified from buffy coats of healthy blood donors was used for deglycosylation studies, by using a variety of glucosidases.

Results: Of three monoclonal antibodies tested i.e. EG2, 614 and 652, the 614 mab was chosen for the experiments. MS analysis of eosinophil extracts demonstrated the presence of ~5 major molecular species of ECP in each subject. ECP from subjects with different ECP 434(G>C) genotypes showed mass differences corresponding to the amino acid shift from arginine to threonine.

ECP purified from buffy coats of healthy blood donors demonstrated an extensive mass heterogeneity with ~10 major molecular species. By the use of a variety of glucosidases it was shown that this heterogeneity was mainly due to N-linked oligosaccharides on which sialic acid, galactose and acetylglucoseamine was positioned.

Conclusions: We conclude that the SELDI-TOF MS technique using specific monoclonal antibodies is a convenient and versatile tool; by means of this technique we could detect both genetic and post-translational causes of the molecular heterogeneity of ECP. This will enable the study of possible molecular modifications of ECP in activated eosinophils obtained from subjects with diseases such as allergy and asthma.

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

POSTER DISCUSSION SESSION I

POSTER #26

EOSINOPHIL CATIONIC PROTEIN (ECP) ACTION AT THE BACTERIA CELL WALL AND CYTOPLASMIC MEMBRANE.

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Background: The eosinophil cationic protein (ECP) is one of main eosinophil protein stored on the eosinophil secondary granules and is released during inflammation processes. It is currently used as a marker for the diagnosis and monitoring of inflammation diseases. ECP together with the eosinophil derived neurotoxin (EDN) belongs to the mammalian ribonucleases family, a family that includes several members with antimicrobial properties that could participate in the innate immune host defense system (Boix and Nogues (2007), *Mol. Biosyst*. 3, 317).

Objectives: Among the described antipathogen cytotoxic activities of ECP, its antibacterial capacity is not shared by its close eosinophil RNase homolog, EDN. We are studying the structural determinants of ECP bactericidal mechanism of action, both at the bacteria wall and cytoplasmic membrane levels.

Methods: Recombinant ECP was expressed and purified using a prokaryote system. ECP was labelled with AlexaFluor 488 fluorophor. We have followed the protein activity on synthetic lipid vesicles by spectrofluorescent methodologies and have characterized the ECP-membrane interaction using confocal microscopy and freeze-fracture cryoelectron microscopy. Affinity binding assays were performed to evaluate the specific association of ECP to lipopolysaccharides (LPS) and its Lipid A component, as well as the interaction to peptidoglycans (PGN). The protein induced bacteria aggregation behaviour, cell morphology and bacteria wall damage have been followed by scanning electron microscopy in both *Escherichia coli* and *Staphylococcus aureus* cell cultures. The protein antimicrobial activity has been tested by a cytoplasmic membrane depolarization.

Results: The results indicate that the protein destabilizes the lipid bilayers by a “carpet-like” mechanism (Torrent et al. (2007) *Biochemistry*, 46, 720). However, the protein destabilization activity on lipid bilayers can only partially account for its bactericidal activity. We have now shown that the protein can bind with high affinity to the bacteria wall components and alters the cell aggregation behaviour. The protein can induce the cytoplasmic membrane depolarization in both Gram-negative and Gram-positive strains.

Conclusions: The results indicate that ECP can destabilize the lipid bilayers by a “carpet –like mechanism” and can bind with high affinity to the bacteria cell wall components. The protein association to the bacteria wall would be the priming step of the protein antimicrobial mechanism of action.

This project was funded by a grant from Ministerio de Educación y Cultura (Grant BMC2003-08485-C02-01) and by the “Fundació La Marató de TV3” (TV3-031110).

POSTER DISCUSSION SESSION I

POSTER #27

EOSINOPHILIC ESOPHAGITIS IS MEDIATED BY A CORTICOSTEROID REVERSIBLE IL-13-STIMULATED KERATINOCYTE TRANSCRIPTOME INVOLVING EOTAXIN-3

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Background: Eosinophilic esophagitis (EE) is an emerging worldwide disease. Early studies have established that esophageal eosinophilia occurs in association with T helper type 2 (Th2) allergic responses, but the mechanism by which this response leads to EE has not been established. We recently identified an EE-specific esophageal transcriptome that included eotaxin-3 as the single most dysregulated gene.

Objectives: In this study, we aimed to uncover the molecular mechanisms involved in the development of EE by focusing on the signaling pathway responsible for the induction of the EE specific transcriptome.

Methods: Esophageal epithelial cells (TE-1, TE-6, TE-7 and TE-13) and primary esophageal epithelial cells were stimulated with IL-13 (0 to 100ng/mL). Transient transfections were performed with plasmids containing luciferase gene driven by eotaxin-3 promoter fragments and a dominant negative form of STAT6. Microarray and Real Time PCR analysis were performed on RNA extracted from primary keratinocytes and normal (NL) and EE esophageal biopsies.

Results: In the current study, we demonstrate that primary and immortalized esophageal keratinocytes are IL-13 receptor positive and markedly produce eotaxin-3 (but not eotaxin-1 and 2) greater than 100-fold following IL-13 stimulation in a dose-dependent manner. IL-13-induced eotaxin-3 mRNA had a 6 hr half-life and was induced by a transcriptional mechanism dependent upon STAT6 and the -89bp proximal STAT6 binding element. Translational studies revealed that IL-13 (but not IL-4) was markedly increased (16-fold) in esophageal biopsies from EE patients compared to normal individuals. Furthermore, IL-13 treatment of keratinocytes was sufficient to induce a global expression transcript profile that remarkably overlapped (22%) with the EE specific esophageal transcriptome, including the induction of eotaxin-3 as the top gene. Lastly, the EE and IL-13-induced transcriptome was largely reversible with glucocorticoid treatment *in vivo*.

Conclusions: Taken together, we propose that the pathogenesis of eosinophilic esophagitis is mediated by an IL-13-stimulated keratinocyte-derived transcriptome (involving eotaxin-3) that is largely reversible with corticosteroid treatment.

This project was funded by grants from NIH (M.E.R.), the Food Allergy and Anaphylaxis Network FAAN (M.E.R.), Campaign Urging Research for Eosinophil Disorders (CURED), the Buckeye Foundation (M.E.R.), The Food Allergy Project (M.E.R.), The American Heart Association (C.B.) and The Thrasher Research Fund NR-0014 (C.B.).

POSTER DISCUSSION SESSION I

POSTER #28

EOSINOPHILS: NEW EFFECTORS IN TUMOR IMMUNITY

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Background: Eosinophils are granulocytes classically involved in helminth parasitic infections and allergic diseases. They can release granule proteins and are also considered as potential immunoregulators. Antitumor response involved several cytotoxic effectors of innate and adaptive immunity. The local inflammatory tumor infiltrate consists mainly in T lymphocytes, macrophages and NK cells, but recent *in vivo* studies have suggested interactions between tumors and eosinophils. Many types of cancer are associated with eosinophilia, either in peripheral blood and/or in the tumor itself, named TATE for “tumor associated tissue eosinophilia”. Although TATE may have favourable prognostic value, little is known however on the exact role played by eosinophils in antitumor responses.

Objectives: Evaluate the tumoricidal activity of eosinophils towards two tumor cell lines, a T lymphoma and a colorectal adenocarcinoma, Jurkat and Colo-205 respectively.

Methods: Eosinophil-mediated cytotoxicity against tumor cell lines is determined by measurement of apoptosis and necrosis induction of target cells using different protocols of flow cytometry, PKH-26-Annexin V-FITC double stainings and FATAL assay.

Results: Our results showed that human eosinophils purified from the peripheral blood of eosinophilic donors are able to induce apoptosis and to kill directly the tumor cell lines *in vitro* using distinct mechanisms. Moreover RT-PCR and flow cytometry revealed for the first time that eosinophils express granzyme A. Experiments are now in progress to define adhesion molecules involved in eosinophil-tumor cell recognition and to identify the respective cytotoxic properties of specific eosinophil cationic proteins *versus* tumoricidal molecules shared with other effector cells, such as granzymes and perforin.

Conclusions: This work provides evidence for new innate immunity mechanisms in eosinophil tumoricidal activity. A better understanding of the role of eosinophils in tumor development might have important therapeutic implications.

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

POSTER DISCUSSION SESSION I

POSTER #29

GENERATION AND CHARACTERIZATION OF POTELLIGENT® ANTI-HUMAN IL-5R-ALPHA CHAIN HUMANIZED MONOCLONAL ANTIBODY, MEDI 563

Masamichi Koike[1], Kazuyasu Nakamura[2], Akiko Furuya[2], Emi Shoji-Hosaka[2], Akihiro Iida [2], Hideharu Anazawa[2], Kenya Shitara[2], Kiyoshi Takatsu[3], and Nobuo Hanai[2]

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Background: Interleukin-5 plays an important role in regulating the differentiation, activation, recruitment and survival of eosinophils. IL-5 signals through a unique IL-5Rα chain expressed on eosinophils, but also basophils and mast cells. and a common β subunit shared with IL-3 and GM-CSF. Therefore, elimination of IL-5 Receptor (IL-5R)$^+$ cells with an antibody is a promising therapeutic strategy for eosinophil-associated aspects of asthma.

Objectives: The purpose of this study was the generation and functional characterization of monoclonal antibodies (Mab) directed against hIL-5Rα generated to exhibit enhanced ADCC function for effective depletion of IL-5Rα bearing cells.

Methods: Mice were immunized with a recombinant hIL-5R protein expressed in baculovirus and CHO cells. Mabs from hybridomas were tested for their ability to inhibit (a) growth of an IL-5 dependent cell line (CTLL-2), (b) IL-5-induced adhesion of human eosinophils and (c) binding of the IL-5 ligand to the hIL-5Rα. One of the Mabs which exhibited the above activities was also tested for antibody-dependent cell-mediated cytotoxic (ADCC) activity. This Mab was produced in fucosyl transferase negative CHO cells (Potelligent®) which have been shown to secrete Mabs completely devoid of fucose and display enhanced ADCC.

Results: One of the best mouse antibodies, (designated as KM1259), was shown to markedly inhibit activities associated with IL-5/ IL-5R functions. In all activities tested the Mab displayed potent inhibition in a dose dependent manner. At a concentration of 1000 ng/ml the Mab inhibited 90% binding of IL-5 to shIL-5R immobilized on plastic wells. Control Mab was only marginally effective, inhibiting only 10%. In the growth inhibition assay, 1000 ng/ml of Mab completely blocked growth of the IL-5-dependent CTLL-2 IL-5R$^+$ cell line. This compared to 18% inhibition for the control Mab. In the eosinophil adhesion assay, 1000 ng/ml Mab again completely ablated adhesion of eosinophils.. The humanized versions of KM1259, created by CDR grafting technology, also showed potent inhibitory activity equivalent to the mouse Mab. The humanized Mabs were further tested for ADCC activity against human eosinophils. It was shown that a humanized fucose-negative Mab induced potent ADCC activity compared to the control fucose-positive Mab. Moreover, while these mAbs exhibited ADCC activity, this occurred in the absence of degranulation and release of the eosinophil toxic granule proteins.

Conclusion: These results indicate that the mouse KM1259 and the humanized hIL-5Rα Mab are biologically active anti-IL-5Rα Mabs that exhibit potent neutralizing activity against IL-5α. The therapeutic potential of the humanized Mab now designated Medi563, is currently under evaluation in a Phase I Trial in mild asthmatics.

POSTER DISCUSSION SESSION II

Animal Models and Patient Studies (Posters 30-64)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
30	**Weller,** Peter	EFFECTS OF MEPOLIZUMAB, AN ANTI-INTERLEUKIN-5 MONOCLONAL ANTIBODY, ON BLOOD EOSINOPHIL COUNTS IN PATIENTS WITH HYPEREOSINOPHILIC SYNDROME	Peter F. Weller, Gerald J. Gleich, William W. Busse and Marc E. Rothenberg, on behalf of the Mepolizumab HES Study
31	**Rosenwasser,** Lanny	CORTICOSTEROID-SPARING EFFECTS OF MEPOLIZUMAB, AN ANTI-INTERLEUKIN-5 MONOCLONAL ANTIBODY, IN PATIENTS WITH HYPEREOSINOPHILIC SYNDROME	Lanny J. Rosenwasser, Lawrence B. Schwartz, Javid Sheikh, Amy D. Klion and Marc E. Rothenberg, on behalf of the Mepolizumab HES Study
32	**Georgelas,** Ann	MEPOLIZUMAB TREATMENT OF THE HYPEREOSINOPHILIC SYNDROME REDUCES SERUM LEVELS OF THE EOSINOPHIL-DERIVED NEUROTOXIN	Ann Georgelas, Lori A. Wagner, Saritha Tunuguntla, Bernie A. Lasalle, Amy Klion, Marc E. Rothenberg, Peter Weller, Hirohito Kita and Gerald J. Gleich
33	**Straumann,** Alex	MEPOLIZUMAB, A HUMANIZED MONOCLONAL ANTIBODY TO IL-5, FOR SEVERE EOSINOPHILIC ESOPHAGITIS IN ADULTS: A RANDOMIZED, PLACEBO-CONTROLLED DOUBLE-BLIND TRIAL	Alex Strauman1, Sebastien Conus, Hirohito Kita, Gail Kephart, Christian Bussmann, Christoph Beglinger, Jatin Patel, Margaret Byrne and Hans-Uwe Simon
34	**Hargreave,** Frederick	PREDNISONE-SPARING EFFECT OF ANTI-IL-5 IN ASTHMA: AN ORIGINAL CASE STUDY	Frederick E. Hargreave and Ann Efthimidais
35	**Hargreave,** Frederick	THE EFFECTS OF ANIT-IL-5 TREATMENT IN PREDNISONE-DEPENDANT EOSINOPHILIC BRONCHITIS ± ASTHMA: A RCT	Kirshnan Parameswaran, Marcia M. Pizzichini, Melanie Kjarsgaard, Ann Efthimiadis, Emilio Pizzichini, Paul O'Byrne and Frederick E. Hargreave
36	**Conus,** Sébastien	ANTI-IL-5 ANTIBODY THERAPY DOES NOT ALTER EOSINOPHIL INFILTRATION OF THE INTESTINAL MUCOSA	Sébastien Conus, Alex Straumann, Eva Bettler, Pascale Grzonka, Ganna Kostylina, Christian Bussmann and Hans-Uwe Simon
37	**Brito-Babapulle,** Finella	EVALUATION OF 28 CASES OF FIP1 L1-PDGFRA NEGATIVE PERSISTENT UNEXPLAINED EOSINOPHILIA WITH EOSINOPHIL END-ORGAN DAMAGE	Finella Brito-Babapulle, Saddon Hassan and Nicholas C.P. Cross
38	**Huss-Marp,** Johannes	USE OF EFALIZUMAB IN HYPEREOSINOPHILIC SYNDROME WITH EOSINOPHILIC DERMATITIS	Johannes Huss-Marp, Rüdiger Hein, Florence Roufosse, Hans-Uwe Simon, Claudia Traidl-Hoffmann, Heidrun Behrendt and Johannes Ring

POSTER DISCUSSION SESSION II

Animal Models and Patient Studies (Posters 30-64)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
39	**Wagner,** Lori	ALEMTUZUMAB THERAPY CAN BE AN EFFICACIOUS TREATMENT FOR REFRACTORY HYPEREOSINOPHILIC SYNDROME	Lori A Wagner, Clarissa J. Christensen, Diane M. Dunn, Gerald J. Spangrude, Ann Georgelas, Linda Kelley, M. Sean Esplin, Robert B. Weiss and Gerald J. Gleich
40	**Ogbogu,** Princess	TREATMENT RESPONSES IN HYPEREOSINOPHILIC SYNDROME: A RETROSPECTIVE, MULTICENTER ANALYSIS	Princess Ogbogu, Bruce Bochner, Joseph Butterfield, Gerald Gleich, Johannes Huss-Marp, Thomas Nutman, Marc E. Rothenberg, Florence Roufosse, Marie-Helene Sajous, Javed Sheikh, Miguel Stein, Andrew Wardlaw, Peter Weller and Amy Klion
41	**Roufosse,** Florence	NEGATIVE PATIENTS RECRUITED IN THE MHE100185 MEPOLIZUMAB CLINICAL TRIAL: PREVALENCE OF L-HES AND DIAGNOSTIC VALUE OF SERUM TARC AMONG FIP1L1-PDGFR	Florence Roufosse, Aurore de Lavareille, Ann Cline, Elie Cogan, Liliane Schandené, Gerald Gleich and Michel Goldman
42	**Squillace,** Diane	MAJORITY OF MAJOR BASIC PROTEIN (MBP) IN BLOOD FROM HYPEREOSINOPHILIC SYNDROME (HES) PATIENTS IS A PRECURSOR FORM, PROMBP	Diane L. Squillace, James L. Checkel, Ayalew Tefferi, Gerald J. Gleich and Hirohito Kita
43	**Collins,** Margaret	FAMILIAL EOSINOPHILIC ESOPHAGITIS (EE)	Margaret H. Collins, Cassie Kirby, Rachel Akers, Ning Wang, Philip E. Putnam, Sean Jameson, Amal Assa'ad, Michael Konikoff, Carine Blanchard, Keith Stringer, J. Pablo Abonia and Marc E. Rothenberg
44	**Fang,** John	COMPARISON OF ESOMEPRAZOLE TO AEROSOLIZED, SWALLOWED FLUTICASONE FOR EOSINOPHILIC ESOPHAGITIS	John C. Fang, Lyska L. Emerson, Kristen Hilden, Kristen L.Thomas and Kathryn A. Peterson
45	**Lampinen,** Maria	DIFFERENT PATTERNS OF EOSINOPHIL ACTIVATION IN CROHN'S DISEASE AND ULCERATIVE COLITIS	Maria Lampinen, Marie Backman, Ola Winqvist, Fredrik Rorsman, Anders Rönnblom, Per Sangfelt and Marie Carlson
46	**Nyenhuis,** Sharmilee	CHANGES IN EOSINOPHIL FUNCTION IN AGING ASTHMA SUBJECTS	Sharmilee M. Nyenhuis, Elizabeth A. Schwantes and Sameer K. Mathur
47	**von Gunten,** Stephan	EXPRESSION OF SIALOSIDE LIGANDS FOR SIGLEC-8 IN HUMAN LUNG AND NASAL POLYPS	Stephan von Gunten, Mary Brummet and Bruce S. Bochner

POSTER DISCUSSION SESSION II

Animal Models and Patient Studies (Posters 30-64)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
48	**Johansson,** Mats	ASSOCIATION BETWEEN P-SELECTIN AND EOSINOPHIL B1 INTEGRIN ACTIVATION IN ASTHMA	Mats W. Johansson and Deane F. Mosher
49	**Redvall,** Elin	ARE THERE DIFFERENCES IN THE INNATE REACTIVITY OF EOSINOPHILS TO AIRBORNE ALLERGENS BETWEEN ATOPIC AND NON-ATOPIC INDIVIDUALS?	Elin Redvall, Ulf Bengtsson and Christine Wennerås
50	**Blensly,** Kirsten	IL-1 MEDIATES THE DELAYED EOSINOPHIL ACCUMULATION AROUND NERVES AND SUBSEQUENT AIRWAY HYPERREACTIVITY AFTER OZONE	Kirsten C. Blensly, Jesse K. Lorton, David B. Jacoby and Allison D. Fryer
51	**Verbout,** Norah	ATROPINE PRETREATMENT ENHANCES EOSINOPHIL ACTIVATION IN AIRWAYS OF ANTIGEN CHALLENGED GUINEA PIGS	Norah G. Verbout, Jesse K. Lorton, David B. Jacoby and Allison D. Frye
52	**Blom,** Kristin	SEQUENCE VARIATIONS OF THE EOSINOPHIL PROTEIN X / EOSINOPHIL DERIVED NEUROTOXIN GENE IN DIFFERENT ETHNICAL POPULATIONS	Kristin Blom, Ulla-Britt Jönsson, Claus M. Reimert, Narcis B. Kabatereine, Francis Kazibwe, Edmund Ireri, Hilda Kadzo, Birgitte J. Vennervald, Per Venge and Lena Håkansson
53	**Jönsson,** Ulla-Britt	THE YIN YANG RELATIONSHIP BETWEEN THE CELLULAR CONTENT OF THE EOSINOPHIL CATIONIC PROTEIN AND THE EOSINOPHIL PROTEIN X / EOSINOPHIL DERIVED NEUROTOXIN IN BLOOD EOSINOPHILS IS RELATED TO THE ECP562 (G>C) AND EPX405 (G>C) POLYMORPHISMS	Ulla-Britt Jönsson, Kristin Blom, Gunnemar Stålenheim and Per Venge
54	**Kottyan,** Leah	THE T-CELL DEATH ASSOCIATED GENE-8 (TDAG8) RECEPTOR IS AN EOSINOPHIL PROTON-SENSING MOLECULE THAT CRITICALLY REGULATES AIRWAY EOSINOPHILIA	L.C. Kottyan, A.R. Collier, K.A. Niese, C.G. Radu, O.N. Witte, M.E. Rothenberg and N. Zimmermann
55	**Hirata,** Hirokuni	ASPIRIN-INDUCED ASTHMA IN LTC4-SYNTHASE TRNSGENIC MICE	Hirokuni Hirata, Masafumi Arima, Takeshi Tokuhisa and Takeshi Fukuda
56	**Brandt,** Eric	A ROLE FOR EOSINOPHILS IN INTESTINAL MAST CELL ACCUMULATION AND ALLERGEN-INDUCED DIARRHEA	Eric B. Brandt, Yoshiyuki Yamada, Heather F. Rainey, Melissa McBride, J. Pablo Abonia and Marc E. Rothenberg

POSTER DISCUSSION SESSION II

Animal Models and Patient Studies (Posters 30-64)

Poster Number	Presenter Name	Abstract Title	Abstract Authors
57	**Wang,** Hai-bin	EOSINOPHILS MEDIATE ALUM IMMUNIZATION-ELICITED B CELL PRIMING	Hai-Bin Wang and Peter F. Weller
58	**McGarry,** Michael	A METHOD FOR THE IN VIVO ASSESSMENT OF EOSINOPHIL-SPECIFIC EFFECTOR FUNCTIONS: COMBINATION OF GENETIC AND ENGRAFTMENT STRATEGIES	Michael P. McGarry, Elizabeth Jacobsen, Ralph Pero, Cheryl Protheroe, Katie O'Neill, Sergei Ochkur, Nancy A. Lee and James J. Lee
59	**Ochkur,** Sergei	CO-EXPRESSION OF IL-5 AND EOTAXIN-2 IN MICE CREATES AN EOSINOPHIL-DEPENDENT MODEL OF RESPIRATORY INFLAMMATION WITH CHARACTERISTICS OF SEVERE ASTHMA	S.I. Ochkur, T. Biechele, E. Jacobsen, B. McElhinney, C. Protheroe, R. Pero, M.T. Borchers, K.R. O'Neill, A.G. Taranova, D.C. Colbert, A. Doyle, M.P. McGarry, Y. Janssen-Heininger, C. Irvin, H.H. Shen, N.A. Lee, and James J. Lee
60	**Mishra,** Anil	ESOPHAGEAL REMODELING DEVELOPS AS A CONSEQUENCE OF TISSUE SPECIFIC IL-5-INDUCED EOSINOPHILIA IN MICE	Anil Mishra, Maiqin Wang, Margaret H. Collins, Philip E. Putnam and Marc E. Rothenberg
61	**Maltby,** Steven	CD34 INVOLVEMENT IN THE DEVELOPMENT OF ALLERGIC ASTHMA AND EOSINOPHIL MIGRATION	Steven Maltby, Marie-Renée Blanchet, D. James Haddon and Kelly M. McNagny
62	**Dharajiya,** Nilesh	ALLERGEN CHALLENGE INDUCES A GENOME-WIDE TH1 TRANSCRIPTOME SIGNATURE	Nilesh Dharajiya, Andrea Estrada, Rafeul Alam and Sanjiv Sur
63	**Rogerio,** Alexandre	ANTI-INFLAMMATORY EFFECTS OF LAFOENSIA PACARI AND ELLAGIC ACID IN A MURINE MODEL OF ASTHMA	Alexandre P. Rogerio, Caroline Fontanari, Alexandre C. Keller, Érica Borducchi, Momtchilo Russo, Edson G. Soares, Deijanira A. Albuquerque and Lúcia H. Faccioli
64	**Simson,** Ljubov	REGULATION OF CARCINOGENESIS BY INTERLEUKIN-5 AND CCL-11: A POTENTIAL ROLE FOR EOSINOPHILS IN TUMOR IMMUNE SURVEILLANCE	Klaus I. Matthaei, Marc E. Rothenberg, Paul S. Foster, Mark J. Smyth, Christopher R. Parish, Ljubov Simson, Julia I. Ellyard and Lindsay A. Dent

POSTER DISCUSSION SESSION II

POSTER #30

EFFECTS OF MEPOLIZUMAB, AN ANTI-INTERLEUKIN-5 MONOCLONAL ANTIBODY, ON BLOOD EOSINOPHIL COUNTS IN PATIENTS WITH HYPEREOSINOPHILIC SYNDROME

Peter F. Weller[1], Gerald J. Gleich[2], William W. Busse[3], Marc E. Rothenberg[4], on behalf of the Mepolizumab HES Study

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Background: Interleukin-5 is important for eosinophil production, differentiation, activity, and survival.

Objective: The efficacy and safety of the anti-interleukin-5 monoclonal antibody, mepolizumab, were assessed in patients with hypereosinophilic syndrome (HES).

Methods: This international, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial involved patients with HES (blood eosinophils >1500/μL for ≥6 months with eosinophilia-related organ involvement or dysfunction and no known cause of eosinophilia), who tested negative for the FIP1L1-PDGFRa fusion gene, and required 20-60 mg/day prednisone monotherapy to maintain blood eosinophils at <1000 cells/μL during a run-in period of ≤6 weeks. Patients received intravenous mepolizumab 750 mg (n=43) or placebo (n=42) every 4 weeks for 36 weeks (final infusion at Week 32). Prednisone was reduced in a stepwise manner according to a predefined algorithm based on eosinophil counts and HES clinical activity.

Results: A total of 41 (95%) patients on mepolizumab vs 19 (45%) on placebo achieved a blood eosinophil count of <600 cells/μL for ≥8 consecutive weeks (P<0.001; odds ratio 18.87 [95% CI: 4.74, 75.17]). This was significant in patients stabilized at baseline on ≤30 mg/day prednisone (28/30 [93%] on mepolizumab vs 18/30 [60%] on placebo; P=0.002), and in patients stabilized on >30 mg/day prednisone (13/13 [100%] on mepolizumab vs 1/12 [8%] on placebo; P<0.001). Mepolizumab was generally well tolerated, with no major safety concerns. One mepolizumab-treated patient died; this was not considered by the investigator to be treatment related.

Conclusions: This study, the largest placebo-controlled trial in HES to date, has shown that mepolizumab maintains blood eosinophils at a low level in FIP1L1-PDGFRa-negative patients with HES and is well tolerated.

This project was funded by a grant from GlaxoSmithKline.

POSTER DISCUSSION SESSION II

POSTER #31

CORTICOSTEROID-SPARING EFFECTS OF MEPOLIZUMAB, AN ANTI-INTERLEUKIN-5 MONOCLONAL ANTIBODY, IN PATIENTS WITH HYPEREOSINOPHILIC SYNDROME

Lanny J. Rosenwasser[1], Lawrence B. Schwartz[2], Javid Sheikh[3], Amy D. Klion[4], Marc E. Rothenberg[5], on behalf of the Mepolizumab HES Study

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Background: Interleukin-5 is important for eosinophil production, differentiation, and survival.

Objective: To evaluate the safety and efficacy of the neutralizing anti-interleukin-5 monoclonal antibody, mepolizumab, in patients with hypereosinophilic syndrome (HES).

Methods: This was an international, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial. Patients had HES (blood eosinophils >1500/μL for ≥6 months with eosinophilia-related organ involvement and no known cause of eosinophilia), tested negative for the FIP1L1-PDGFRA fusion gene, and required 20-60 mg/day of prednisone monotherapy to maintain blood eosinophils at <1000 cells/μL during a run-in period of ≤6 weeks. Patients received intravenous mepolizumab 750 mg (n=43) or placebo (n=42) every 4 weeks for 36 weeks (final infusion at Week 32). The prednisone dose was tapered according to a predefined algorithm based on eosinophil counts and HES clinical activity.

Results: Overall, 36 (84%) mepolizumab-treated patients vs 18 (43%) placebo-treated patients achieved the primary endpoint (≤10 mg/day prednisone for ≥8 consecutive weeks) (P<0.001; odds ratio 8.01 [95% CI: 2.69, 23.78]). Time to achievement of the primary endpoint, a post-hoc analysis, was significantly shorter in mepolizumab- vs placebo-treated patients (P=0.002). All other steroid-sparing endpoints (including the proportion of patients prednisone-free for ≥1 day) were statistically significant in favor of mepolizumab. Mepolizumab was generally well tolerated; no major safety concerns were observed. One patient receiving mepolizumab died, but this was considered by the investigator to be unrelated to treatment.
Conclusions: This study, the first and largest placebo-controlled trial in patients with HES to date, shows that mepolizumab is well-tolerated and offers clinical benefit in terms of steroid sparing/withdrawal in FIP1L1-PDGFRA-negative patients with HES.

This project was funded by a grant from GlaxoSmithKline.

POSTER DISCUSSION SESSION II

POSTER #32

MEPOLIZUMAB TREATMENT OF THE HYPEREOSINOPHILIC SYNDROME REDUCES SERUM LEVELS OF THE EOSINOPHIL-DERIVED NEUROTOXIN

Ann Georgelas[1], Lori A.Wagner[1], Saritha Tunuguntla[1], Bernie A. Lasalle[1], Amy Klion[2], Marc E. Rothenberg[3], Peter Weller[4], Hirohito Kita[5] and Gerald J. Gleich[1]

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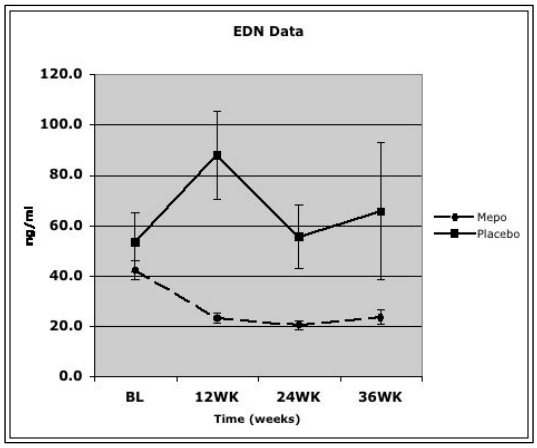
Background: Mepolizumab is an effective treatment for *FIP1L1/PDGFRA*-negative patients with the hypereosinophilic syndrome (HES) by reducing blood eosinophils and by reducing glucocorticoids required for HIS control. The eosinophil-derived neurotoxin (EDN) is localized in the granule matrix, is a potent ribonuclease (also referred to as RNase2) and is a neurotoxin producing the Gordon phenomenon. EDN is released by stimulated eosinophils and deposited at sites of tissue damage. Thus it marks eosinophil participation in disease activity and tissue damage.

Objectives: To determine whether EDN levels are altered by mepolizumab treatment of patients with HES and whether differences exist between the treated and control patients.

Methods: Sera were collected from study patients at four different time points, baseline, 12, 24 and 36 weeks, respectively. EDN was assayed using chemiluminescence immunoassay technology and two monoclonal antibodies. Briefly, sera were incubated in plates coated with J167-6C5 capture antibody, washed and incubated with acridinium labeled J167-2G4 anti-EDN detection antibody. After a final wash, EDN was measured using a LMAX II^{384} luminometer and the SOFTmax® PRO (ver.4.6) Software.

Results: EDN serum levels (mean+/- SEM) in the mepolizumab and placebo patients are shown in the figure. In mepolizumab patients EDN levels significantly dropped between baseline and weeks 12, 24 and 36 ($p=0.001$ for all time points). In the placebo patients EDN levels significantly rose between baseline and week 12 ($p=0.0150$) and week 36 ($p=0.03$). Between the groups EDN levels differed from baseline at all time points ($p<0.001$).



Conclusions: Mepolizumab treatment reduced EDN serum levels compared to the placebo treated patients. This reduction is consistent with an ability of mepolizumab to suppress eosinophil infiltration and the release of toxic eosinophil granule proteins in tissues of HES patients.

This project was funded by grants from the National Institute of Allergy and Infectious Diseases, AI061097 and AI34486.

POSTER DISCUSSION SESSION II

POSTER #33

MEPOLIZUMAB, A HUMANIZED MONOCLONAL ANTIBODY TO IL-5, FOR SEVERE EOSINOPHILIC ESOPHAGITIS IN ADULTS: A RANDOMIZED, PLACEBO-CONTROLLED DOUBLE-BLIND TRIAL

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Background: Eosinophilic Esophagitis (EE) is a clinico-pathological condition defined by proton-pump-inhibitor refractory esophagus-related symptoms in combination with a dense esophageal eosinophilia. IL-5 is the major cytokine responsible for the differentiation, recruitment, and activation of eosinophils. Mepolizumab (Mepo) binds specifically to and inactivates IL-5.

Objectives: This phase 2a translational study evaluated the tolerability, efficacy and pharmacokinetics of Mepo in adults with severe EE, unresponsive to or dependent on corticosteroids.

Methods: Eleven adults with active EE (>20 eos/hpf and dysphagia) were randomized to Mepo 750mg (5, mean age 32 yr) or placebo (Pbo) (6, mean age 34 yr) by intravenous infusion at days 0 and 7. Those not in complete remission at week (Wk) 4 (<5 eos/hpf) received 2 further doses 4 weeks apart, of 1500mg Mepo or Pbo per their original randomization, and repeat biopsy 4 weeks post final infusion. Follow-up continued for 21 weeks. From the run-in period (weeks 0 to 4) and throughout the study, subjects received no other anti-eosinophil therapy. Pre- and post-treatment disease activity was assessed clinically, endoscopically, histologically and via biomarkers of inflammation and eosinophil activity. Subjects completed a symptom diary up to Wk 25.

Results: Treatment with Mepo was well tolerated and no clinically relevant adverse events occurred. A convincing decrease in mean blood eosinophils occurred with Mepo (0.54 GI/L at baseline to 0.08 at Wk 4), but not with Pbo (0.47 to 0.43) consistent with previous studies. In the esophageal tissue a similar major reduction in mean eosinophil count was observed – 82/hpf at screen to 27 at Wk 4 (-67%) in the Mepo group and from 61 to 45 (-25%) in the Pbo group, although the primary endpoint of peak eosinophils of <5/hpf was not achieved. No further changes in eosinophil numbers were observed at Wk 13 in either group. We also obtained evidence for reduced eosinophil degranulation both in blood and esophageal tissues. Mepo treatment was not associated with a decline of infiltrating T cells and mast cells. The treatment-related changes in eosinophil numbers were associated with much improvement of the swallowing difficulties in 2 subjects at 2 months post last infusion of Mepo, whereas only 1 Pbo-treated subject reported improvement.

Conclusions: Treatment of adults with severe EE with Mepo is well tolerated. The blockade of IL-5 resulted in a highly significant decrease of eosinophil numbers in blood and esophageal tissues. These decreases were accompanied by clinical improvement in a subgroup of subjects.

POSTER DISCUSSION SESSION II

POSTER #34

PREDNISONE-SPARING EFFECT OF ANTI-IL-5 IN ASTHMA: AN ORIGINAL CASE STUDY

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Background: Humanized anti-IL-5 monoclonal antibody is effective in reducing blood and tissue eosinophils and producing clinical improvement in patients with hypereosinophilic syndrome (HES). However, when applied to the allergen inhalation model, while it inhibited sputum eosinophilia, it did not inhibit the late asthmatic response.

Objectives: We examined the prednisone-sparing effect of anti-IL-5 in a patient with HES (affecting colon and skin) who also had asthma and rhinosinusitis.

Methods: The patient was investigated in detail, seen every 1-4 weeks depending on circumstances, monitored by symptoms, spirometry, quantitative sputum cell counts and blood eosinophils, and subjected to open trials of treatment which eventually included anti-IL5.

Results: The patient, a 32-year woman, was referred in 2004. Six years earlier she began to have symptoms of very mild asthma and hay fever. For 3-years she had HES and more severe asthma which required daily treatment with a minimum dose of prednisone 35-40 mg daily in addition to budesonide 800 ug twice daily and formoterol 24 ug twice daily. Before starting prednisone, her blood eosinophils were 7.4 x 10^9L and pANCA was normal; there was no vasculitis, leukemia or parasitic infection. She was mildly atopic. Trials of treatment with hydroxyurea, cyclophosphamide, interferon and cyclosporin did not allow any reduction of prednisone without exacerbations of eosinophilic bronchitis and asthma. Subsequent treatment with anti-IL-5 750 mg IV at intervals chiefly at 1-month for 14 doses allowed a reduction in prednisone to 10 mg daily without blood or sputum eosinophilia or deterioration in the control of her asthma or other system involvement. Then, while still on treatment, the eosinophilic bronchitis recurred and, when stopping anti-IL-5, required higher doses of corticosteroids than before the start of anti-IL-5.

Conclusions: Anti-IL-5 had a dramatic prednisone-sparing effect but subsequently appeared to lose its effect in association with the development of more relative steroid-resistance than to begin with.

This study was unfunded.

POSTER DISCUSSION SESSION II

POSTER #35

THE EFFECTS OF ANIT-IL-5 TREATMENT IN PREDNISONE-DEPENDANT EOSINOPHILIC BRONCHITIS ± ASTHMA: A RCT

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Background: Anti-IL-5 is known to reduce blood and tissue eosinophils and to be clinically effective in hypereosinophilia syndrome (HES). Its clinical efficacy in asthma has not been demonstrated but in a patient with HES with asthma we observed a pronounced prednisone-sparing effect.

Objectives: To investigate the effects of anti-IL-5 (mepolizumab) treatment on sputum eosinophilia, asthma control and minimum prednisone and inhaled corticosteroid doses in patients without HES but with eosinophilic bronchitis with or without asthma.

Methods: A single centre double blind randomized placebo controlled parallel group trial. 20 adult patients with prednisone-dependant eosinophilic bronchitis with or without asthma were recruited when their minimum dose to prevent frequent clinical exacerbations was established. This dose was reduced if necessary to allow a recurrence of sputum eosinophilia. Treatment with mepolizumab 750 mg or placebo intravenously was begun and repeated another 4 times at intervals of 1-month. The patients were seen every 2-weeks. After 4 weeks prednisone was reduced by a daily dose of 5 mg (in those on >10mg) or 2.5 mg (those on ≤10mg) at the monthly visits until the patient had completed the 5 doses, or there was a clinical exacerbation of eosinophilic bronchitis, or steroid-withdrawal effects were of concern. The study ended in each patient 8-weeks after the last dose of test medication. The variables measured included asthma control questionnaire, symptoms severity by Likert scale, asthma specific quality of life, spirometry, PC20 methacholine, quantitative sputum cell counts, exhaled NO and blood eosinophils. The primary outcome will be the corticosteroid-sparing effect of mepolizumab.

Results: The last patient will end the study on 27 June. Once the database has been checked, the results will be analyzed and discussed at the Symposium.

This project was funded by a grant from GlaxoSmithKline.

POSTER DISCUSSION SESSION II

POSTER #36

ANTI-IL-5 ANTIBODY THERAPY DOES NOT ALTER EOSINOPHIL INFILTRATION OF THE INTESTINAL MUCOSA

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Background: Under healthy conditions, the only non-haematopoietic organ showing a relevant number of tissue-dwelling eosinophils is the mucosa of the gastrointestinal tract. IL-5 is a crucial T_H2 cytokine and responsible for the differentiation, recruitment and activation of eosinophils. Mepolizumab (Mepo) binds specifically to and inactivates IL-5, resulting in reduced eosinophil differentiation when applied to eosinophilic patients.

Objectives: The purpose of this study was to examine the effect of Mepo on physiological eosinophil infiltration of the intestinal mucosa of eosinophilic esophagitis (EE) patients.

Methods: Intestinal biopsies were taken within a randomized, placebo-controlled double-blind trial using Mepo in patients suffering from active EE without gastrointestinal involvement. Eleven adults with active EE (>20 eos/hpf and dysphagia) were randomized to Mepo 750 mg (5, mean age 32 yr) or placebo (Pbo) (6, mean age 34 yr) by intravenous infusion at days 0 and 7. Those not in complete remission at week (Wk) 4 (<5 eos/hpf) received 2 further doses 4 weeks apart, of 1500 mg Mepo or Pbo per their original randomization, and repeat biopsy 4 weeks post final infusion. Follow-up continued for 21 weeks. From the run-in period (weeks 0 to 4) and throughout the study, subjects received no other anti-eosinophil therapy. Intestinal infiltrating immune cells were identified by immunofluorescence analysis.

Results: A decrease in mean blood eosinophils occurred with Mepo (0.54 GI/L at baseline to 0.08 at Wk 4), but not with Pbo (0.47 to 0.43) consistent with previous studies. Similarly, we detected a significant reduction in mean eosinophil count in the esophageal tissues (85.1/hpf at screen to 32.3 at Wk 4 in the Mepo group and 46.9/hpf to 47.0/hpf in the Pbo group). In contrast, no decline of eosinophil numbers was detected in intestinal tissues as a consequence of Mepo treatment (17.9/hpf to 16.1 for Mepo and 17.7/hpf to 17.1 for Pbo). Moreover, Mepo treatment did also not reduce physiologically infiltrating T cell and mast cell numbers in these tissues.

Conclusions: The therapeutic blockade of IL-5 with Mepo does not alter the physiologic infiltration of the intestinal mucosa with eosinophils, T cells, and mast cells. Therefore, it is unlikely that Mepo therapy causes intestinal immune dysbalance.

POSTER DISCUSSION SESSION II

POSTER #37

EVALUATION OF 28 CASES OF FIP1 L1-PDGFRA NEGATIVE PERSISTENT UNEXPLAINED EOSINOPHILIA WITH EOSINOPHIL END- ORGAN DAMAGE

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Background: In 1997 the term eosinophil end-organ damage was introduced to facilitate management of patients with high eosinophil counts in whom damage to organs such as heart, lungs, and skin by eosinophils was associated with persistent unexplained eosinophilia (no obvious cause for eosinophilia was identified). In 2003 Cools et al identified the presence of the FIP1L1-PDGFRA gene as the causation of eosinophilia in 50% of cases of hypereosinophilic syndromes with end organ damage. Cases which are negative appear to form a distinctive group whose aetiopathogenesis and clinical significance appear unclear.

Objectives: To identify the extent of end organ damage in FIP1L1-PDGFRA negative cases and/or identify rarer causes of unexplained eosinophilia in all cases of eosinophilia presenting from 14.06.2002 to 14.06.2006. Eosinophilia had to be present on two separate blood tests done I month apart with eosinophil counts >1-1.5 x 10 9/l.

Methods: All cases of unexplained eosinophilia (Eosinophil count greater than 1.5 x 10 9/L) seen at our institution in a 4 year period in whom no obvious cause of eosinophilia was found were evaluated for the presence of the FIP1L1-PDGFRA gene, TEL-PDGFRB and variant BCR-ABL gene. Of 40 cases seen 2 men were FIP1L1-PDGFRA positive, 3 others (2 men and 1 woman) had myeloproliferative diseases, I man had clozapine induced eosinophilia, one woman Kimura Weils disease and 1 man had cutaneous mastocytosis .There were 28 cases(14 women ,2 children,12 men) who were FIP1L1 – PDGFRA negative and had persistent unexplained eosinophilia without clonal T cells. These cases were followed up over a 4 year period and tested serially for damage to end organs by eosinophils.FIP1L1-PDGFRA was tested and found negative on two separate occasions. Ig E levels had to be less then 500 iu/l to exclude allergy and a therapeutic trial of mebendazole 100mg daily for three days was given in all cases even if parasite testing did not identify a parasitic infestation.

Results: There were 28 cases (14 women, 2 children,12 men) who were FIP1L1-PDGFRA negative which contrasts with the Male Female ratio seen in FIP1 L1-PDGFRA positive cases in which males predominate. The age ranged from 9-80. Four year follow up identified a chronic illness in these cases with no damage to vital organs. Clinical symptoms complained of included fatigue(28), joint and muscle pains(28),skin rashes(10), blackouts(1), thrombotic events(2), diarrhoea(2), fasciitis(2), gastroenteritis(2), cough(2), and breathlessness(2).No organomegaly was seen. In one clonal T cells were identified on serial testing after 1 year of follow up.

Conclusions: Cases of FIP1L1-PDGFRA negative hypereosinophilia are distinct group with a chronic illness mainly affecting skin and joints who respond well to symptomatic treatment, oral steroids, or hydroxyurea.

POSTER DISCUSSION SESSION II

POSTER #38

USE OF EFALIZUMAB IN HYPEREOSINOPHILIC SYNDROME WITH EOSINOPHILIC DERMATITIS

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Background: In spite of recent advances in treatment of hypereosinophilic syndrome (HES), eosinophilic dermatitis in HES continues to constitute a therapeutic problem, and skin inflammation and pruritis are often resistant to the variety of agents proposed as therapeutic options. LFA1/CD11a was reported as a possible adhesion-related therapeutic target on the surface of eosinophils and lymphocytes, influencing the influx of inflammatory cells in the tissue. The humanized monoclonal IgG1-antibody efalizumab, directed against the CD11a-chain of LFA1 is approved for psoriasis, but no data on its usefulness in HES is available.

Objectives: It was the aim of this pilot trial to evaluate the use of efalizumab in patients with HES and eosinophilic dermatitis.

Methods: Three patients meeting the diagnostic criteria for HES were treated with efalizumab between October 2005 and May 2007. The patients were male, aged 41 (I), 60 (II), and 62 (III) years, respectively, had severe skin involvement and were FIP1L1/PDGFRA negative. Patient I had a proven lymphocytic variant of HES with 70.9% CD3 negative CD4 positive T-cells, whereas immunophenotyping revealed no abnormalities in patients II and III who were diagnosed with idiopathic HES. All patients received efalizumab 1mg/kg body weight s.c. once weekly. To investigate efficacy, the severity of skin involvement was assessed, pruritis was measured by visual analog scale, and skin biopsies were taken at different time points. Besides eosinophil counts, immunophenotyping was performed and cytokines were analyzed.

Results: In patient I efalizumab led to significant improvement of skin involvement and reduction of pruritis within 4 weeks. The changes were reflected by a decrease of the inflammatory infiltrate and a reduction of eosinophils in the skin sample. Due to the favorable effect, treatment intervals were extended to two weeks and therapy has now been ongoing for more than 1,5 years. Detailed analysis of CD3 negative CD4 positive T-cells of patient I revealed the following profile: TCRa/b negative, TCRg/d negative, CD45RO positive, 32% CD25 positive, CD95 positive, CD2 positive, 10% HLA-DR positive, CD7 negative, CD27 negative, and CD5 high. Intracellular cytokines on gated CD3 negative CD4 positive T-cells were: IL-2 54%, IL-4 44%, IL-5 39%, and IL-13 60.5%. In patients II and III the treatment response was less favorable, leading to no improvement of skin condition and pruritis. Efalizumab therapy was interrupted in patients II and III after 11 and 5 weeks, respectively. Detailed analysis of skin and blood samples of these patients is currently ongoing.

Conclusions: In the course of a pilot trial on efalizumab in three FIP1L1/PDGFRA negative patients with HES and eosinophil dermatitis, the drug proved efficient in one patient with lymphocytic variant HES, whereas there was no improvement in the two patients with idiopathic HES. This effect might be due to changes in epidermotrophism of both eosinophils and pathogenic T-cells after efalizumab treatment. Further investigations on additional patients included in the trial are needed.

This project was supported by a grant from Merck Serono, Unterschleißheim, Germany.

POSTER DISCUSSION SESSION II

POSTER #39

ALEMTUZUMAB THERAPY CAN BE AN EFFICACIOUS TREATMENT FOR REFRACTORY HYPEREOSINOPHILIC SYNDROME

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Background: Hypereosinophilic Syndrome (HES) is a proliferative disease of eosinophils that results in organ damage. Classical treatments for the disease include corticosteroids, hydroxyurea and interferon-alpha. HES can be caused by a FIP1L1-PDGFRA fusion that results in enhanced kinase activity and can be controlled by imatinib mesylate (Gleevec). However, substantial proportions of patients do not have this gene fusion and cannot be treated with Gleevec. Overproduction of IL-5, resulting in eosinophil proliferation and activation is also a symptom of this disease. Therefore, the disease may be treated with mepolizumab, a monoclonal anti-IL-5 antibody. Alemtuzumab (Campath), a monoclonal antibody against CD52, which has been reported to be present on the surface of eosinophils but not neutrophils, has been used to treat refractory HES in this case report.

Objectives: To treat a patient with HES and microinfarcts in the brain who has not responded to Gleevec, mepolizumab or corticosteroids and only partially to alpha interferon and hydroxyurea.

Methods: The patient was treated biweekly with a 30 mg injection of alemtuzumab weekly or biweekly.

Results: The patient's blood levels of eosinophils went from approximately 7000 per ul on alpha interferon treatment to less than 500 per ul on Alemtuzumab treatment (Figure 1). The patient also reported greater mental clarity and energy levels than during prior treatment.

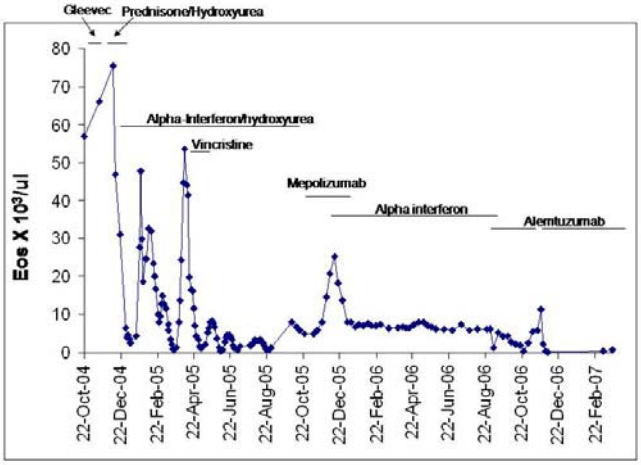

Figure 1. Blood eosinophil levels following various treatments including alemtuzumab.

Conclusions: Alemtuzumab treatment may be used in patients that do not respond to other therapies.

POSTER DISCUSSION SESSION II

POSTER #40

TREATMENT RESPONSES IN HYPEREOSINOPHILIC SYNDROME: A RETROSPECTIVE, MULTICENTER ANALYSIS

Princess Ogbogu[1], Bruce Bochner[2], Joseph Butterfield[3], Gerald Gleich[4], Johannes Huss-Marp[5], Thomas Nutman[1], Marc E. Rothenberg[6], Florence Roufosse[7], Marie-Helene Sajous[2], Javed Sheikh[8], Miguel Stein[6], Andrew Wardlaw[9], Peter Weller[8], and Amy Klion[1]

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Background: Hypereosinophilic syndrome (HES) is a heterogeneous group of rare disorders defined by the presence of persistent peripheral blood eosinophilia >1500mm3, the absence of a secondary cause, and evidence of eosinophil-associated end organ damage. Although a wide variety of agents have been used for the treatment of HES, published therapeutic trials have been restricted to case reports and small case series. This is due, in large part, to the lack of sufficient numbers of affected patients.

Objectives: The purpose of the present study was to assemble a large, multicenter database of the baseline clinical characteristics and responses to treatment of patients with HES and to use these data to assess the efficacy of the various therapies and the factors predictive of specific treatment responses.

Methods: Patients meeting diagnostic criteria for HES, evaluated between January 2001 and December 2006 at nine participating institutions with expertise in the evaluation of eosinophilic disorders, were included in the study. Clinical and laboratory data pertaining to baseline characteristics and treatment responses were collected retrospectively, entered without identifiers into an Excel database and compiled for analysis. Potential duplicates were removed on the basis of a combination of factors, including birth year, gender, and clinical features.

Results: Data from 158 patients, 93 men and 67 women, were analyzed. Median age was 52 years (range 6-84 years). Corticosteroids were the most frequently used therapy, with 152 patients treated and a response rate of 61% (93/152) at maximal doses ranging from 10-60 mg of prednisone daily. The reported response rate to interferon-alpha (5/41 patients or 12%) was lower than expected; however, side effects of therapy were common and led to discontinuation of therapy in a majority of patients. In contrast, hydroxyurea and cyclosporine were well tolerated, but had low efficacy (response rates of 21% and 20%, respectively). Imatinib was effective in only 19/59 (32%) patients treated; however, the response rate was dramatically higher (12/13 or 92%) in those with the FIP1L1/PDGFRA mutation. Finally, although monoclonal anti-IL5 therapy was tried in 52 patients, efficacy data are not available since the most common reason for discontinuation was the completion of the research trial. A variety of other agents were used in small numbers of patients with HES, including methotrexate (n=12), mycophenolate mofetil (n=6), azathioprine (n=4) and dapsone (n=4). Analysis of clinical and laboratory predictors of response to the various treatments is ongoing.

Conclusions: Retrospective multicenter analysis can be a useful tool for the assessment of treatment responses in rare disorders. The identification of promising therapies and/or factors predictive of treatment response in HES should provide a basis for the rational design of future treatment studies.

POSTER DISCUSSION SESSION II

POSTER #41

PREVALENCE OF L-HES AND DIAGNOSTIC VALUE OF SERUM TARC AMONG FIP1L1-PDGFRα NEGATIVE PATIENTS RECRUITED IN THE MHE100185 MEPOLIZUMAB CLINICAL TRIAL

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Background: Patients fulfilling diagnostic criteria of HES may fall into distinct pathogenic categories, including FIP1L1-PDGFRα-associated disease, and lymphocytic-HES (L-HES). In the latter variant, hypereosinophilia results from increased IL-5 production by T cell subsets, which are often monoclonal and display aberrant surface phenotypes, most frequently $CD3^-CD4^+$. Identification of such patients may guide therapy, through preferential administration of agents that target T cells, and is essential for early detection of T cell lymphoma that may develop several years after diagnosis of HES.

Objectives: We took advantage of widespread HES patient recruitment (n=85) afforded by the international randomized double-blind, placebo-controlled clinical trial investigating efficacy of mepolizumab in F/P^- patients (MHE100185), to assess prevalence of L-HES in this population, and to determine whether increased serum thymus and activation regulated chemokine (TARC) represents a robust diagnostic bio-marker for L-HES.

Methods: Serum was collected at day 1, weeks 12, 24, and 36/EW, from 82 patients included in MHE100185, for measurement of serum TARC levels using the commercial RnD ELISA kit. Additionally, for patients providing specific consent for exploratory analyses, an EDTA tube was drawn for lymphocyte phenotyping by flow cytometry, staining for CD3, CD4, CD8, CD2, CD5, CD7, CD25, HLA-DR, and TCRα/β.

Results: T cell flow studies were available for 67 patients included in the clinical trial (44 USA, 23 Europe). $CD3^-CD4^+$ T cells were detected in 8 patients (4 USA, 4 Europe), with percentages ranging from 0.6 to 92% of gated lymphocytes. Low CD7 expression, high CD5 expression, and high CD25 expression compared to $CD3^+CD4^+$ cells, indicated that the $CD3^-CD4^+$ subset was indeed a distinct population. In addition, one patient with a clonal $CD4^+CD7^{dim}$ population was identified. Among the 82 patients for whom serum TARC levels were available, 20 had levels exceeding 2000 pg/ml at least at one time-point during the course of the study (normal values : mean 265 pg/ml, range 66-625, in a series of 38 healthy subjects). Serum TARC levels > 5000 pg/ml were found in all patients with $CD3^-CD4^+$ T cells, including one patient with only 0.6% abnormal T cells (n=8). For the remaining 12 patients with increased TARC, $CD4^+CD7^{dim}$ cells were detected in one, flow data was not available (n=3), or showed no reproducible abnormalities (n=8).

Conclusions: Among F/P^- patients recruited for the MHE100185 study, 12% had a $CD3^-CD4^+$ T cell subset, and 1 had a $CD4^+CD7^{dim}$ T cell clone. Serum TARC levels were >5000 pg/ml in all patients with $CD3^-CD4^+$ L-HES, making it a sensitive marker for diagnosis of this variant. Its specificity remains to be assessed by further studies including TCR rearrangement patterns and IL-5 measurement in culture supernatants.

This project was supported by GlaxoSmithKline and funded by a grant from the Belgian National Fund for Scientific Research.

POSTER DISCUSSION SESSION II

POSTER #42

MAJORITY OF MAJOR BASIC PROTEIN (MBP) IN BLOOD FROM HYPER-EOSINOPHILIC SYNDROME (HES) PATIENTS IS A PRECURSOR FORM, proMBP.

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Background: During eosinophil differentiation, eosinophil granule major basic protein (MBP) is made as a 32-kDa precursor form (i.e. proMBP), which is then processed by protease(s) to the 14-kDa mature MBP (i.e. MBP). It is thought that the pro-piece in proMBP protects the developing eosinophil from toxic MBP during granule processing. It is unknown whether "MBP" detected in biological fluids is MBP or proMBP. **Objectives:** We wanted to quantitate MBP and proMBP in serum from patients with HES and other myeloproliferative disorders.
Methods: Mice were immunized with native MBP or native proMBP and positive clones were expanded. Capture and detection monoclonal antibody pairs specific for either MBP or proMBP were screened and selected by radioimmunoassay (RIA), using native MBP and recombinant proMBP, respectively. Serum samples from patients with HES and other myeloproliferative disorders were reduced, alkylated and analyzed for MBP and proMBP simultaneously using matched pair monoclonal antibodies and RIA. Western blot analyses were performed on selected samples to corroborate findings of the RIA.
Results: Titrations of proMBP and MBP in the RIAs for both proMBP or MBP showed less than 1% crossreactivity between these assays. Serum concentrations of MBP and proMBP, as measured by RIA, were as follows:

Patients (n)	MBP median	MBP range	MBP % abnormal*	proMBP median	proMBP range	pro MBP % abnormal*
Normal (28)	<4	<4-26	4%	101	11-290	4%
HES (36)	72	<4-696	64%	3726	50-22000	89%
CML (16)	<4	<4-84	9%	247	26-4624	50%
ET (19)	<4	<4-14	0%	114	33-560	11%
MDS (21)	<4	<4-180	5%	288	17-22000	57%
MMM (89)	<4	<4-63	1%	320	42-6392	61%
PV (41)	<4	<4-48	2%	152	20-3904	32%
SMCD (18)	<4	<4-50	11%	156	42-3468	39%

Data are presented as ng/ml, * abnormal values: MBP >14 ng/ml, proMBP >234 ng/ml.
CML chronic myelogenous leukemia, ET essential thrombocythemia, MDS myelodysplastic syndrome, MMM myelofibrosis with myeloid metaplasia, PV polycythemia vera, SMCD systemic mast cell disease.

Furthermore, by western blot, MBP was undetectable in normal controls and present in a few HES patients; proMBP was detected in both normal and HES sera. Finally, by RIA, nasal secretions from patients with chronic rhinosinusitis (as a control) contained more MBP (median 1399 ng/ml) than proMBP (median 13 ng/ml).
Conclusions: The majority of serum "MBP" in HES patients, as well as normal controls, is proMBP, rather than MBP. Sera from some other myeloproliferative disorders also contain proMBP, suggesting the potential involvement of eosinophil precursors. The proMBP may be useful to differentiate the myeloid and lymphocytic origin of eosinophilia.

This project was funded by grants from the NIH (AI09728, AI34486).

POSTER DISCUSSION SESSION II

POSTER #43

FAMILIAL EOSINOPHILIC ESOPHAGITIS (EE)

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Background: EE is often associated with atopic sensitization, which may be familial. We have reported families with multiple members who have EE, but the extent of familial clustering and the familial phenotype have not been reported.

Objectives: Report our experience with familial EE, and compare characteristics of familial to sporadic EE patients.

Methods: Pathology and clinical databases were searched for EE patients who had other family members with EE; one member of each family was matched for gender and age to EE patients who do not have affected family members.

Results: Fifty-nine members (69% male) of 26 families were mean age 10.3 years when biopsy-proven EE was diagnosed. A parent of a male patient had EE in 4 families. The most common complaint at diagnosis was dysphagia (68%), the most common endoscopic finding was mucosal furrows (93%), asthma was prevalent (51%), and skin prick tests were positive in most familial EE patients (>70%). Twenty-six patients who had sporadic EE and were matched for date of birth and gender to one member of each EE family did not differ clinically, endoscopically or histologically from familial EE patients, except that in patients who had mucosal furrows familial EE patients had a lower peak eosinophil count in the distal esophagus (P = 0.03). The only recorded ethnic origin for familial or sporadic patients was Caucasian.

Conclusions: These data support a familial pattern of inheritance of EE. Mucosal furrows in familial EE patients may have a different pathogenesis from furrows in sporadic EE patients. EE should be strongly suspected in symptomatic family members of EE patients, especially in Caucasian families.

POSTER DISCUSSION SESSION II

POSTER #44

COMPARISON OF ESOMEPRAZOLE TO AEROSOLIZED, SWALLOWED FLUTICASONE FOR EOSINOPHILIC ESOPHAGITIS

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Background: Eosinophilic esophagitis (EoE) is an increasingly recognized condition characterized by dysphagia, food impaction and/or chest pain in predominantly young to middle-aged males. The endoscopic exam includes multiple concentric rings, vertical furrowing, or subtle strictures. It is characterized histologically by infiltration of eosinophils into the esophageal squamous epithelium or deeper tissues. The pathogenesis of EE is unclear but both GERD and allergy/atopy have been implicated. Retrospective studies have demonstrated improvement both with swallowed topical corticosteroids and proton pump inhibitors. There have been no prospective studies comparing treatment of EoE with acid suppression using proton pump inhibitors vs. topical corticosteroids.

Objectives: To determine the outcome of adult EoE patients treated with esomeprazole vs. swallowed fluticasone. Secondary aims were to determine the prevalence of GERD in EoE patients and change in eosinophil infiltration with treatment.

Methods: Prospective randomized controlled trial of adult (age 18-80) patients diagnosed with EoE by symptoms of dysphagia, food impaction and/or chest pain and endoscopic biopsies demonstrating ≥ 15 eos/hpf averaged over at least 5 hpf's. The subjects underwent pH study and were randomized to esomeprazole (40 mg PO qAM) or aerosolized, swallowed fluticasone (440mcg PO BID) for 8 weeks. EGD with biopsy was repeated at 8 weeks. Dysphagia and validated GERD scales were obtained at baseline and 8 weeks. Wilcoxon signed rank and rank sum tests were used to compare dysphagia scores and eosinophil concentrations within and between groups.

Results: 30 subjects were enrolled into the study, 5 were lost to follow-up before repeat EGD. Mean age was 37 years (range 18-79), 23/30 (77%) were male. 25/30 (83%) had acid reflux by pH study. Both treatment groups significantly decreased their dysphagia score and eosinophil infiltration compared to baseline. There was no significant difference in decrease in dysphagia score and eosinophil infiltration between the two groups (p=0.35, p=0.70).

Conclusions: GERD is common in adult patients with EoE. EoE patients significantly improve their dysphagia and decrease esophageal eosinophilic infiltration with either treatment. There was no difference in improvement in dysphagia or eosinophil infiltration in patients treated with topical fluticasone or oral proton pump inhibitors.

This project was funded by a grant from the American Society for Gastrointestinal Endoscopy (ASGE)

POSTER DISCUSSION SESSION II

POSTER #45

DIFFERENT PATTERNS OF EOSINOPHIL ACTIVATION IN CROHN'S DISEASE AND ULCERATIVE COLITIS

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Background: The role of eosinophil granulocytes in inflammatory bowel disease (IBD) is largely unknown. These cells are normally present in the intestinal mucosa participating in the host defence, but the number of eosinophils is highly increased in patients with IBD, and eosinophil cytotoxic proteins have been found in faeces from patients with active disease. However, little is known about the activity and survival of eosinophils in different stages of disease, and about the differences that may exist between Crohn's disease (CD) and ulcerative colitis (UC) in this respect.

Objectives: The aim of this study was to compare eosinophil activity and survival in different stages of Crohn's disease and ulcerative colitis.

Methods: Biopsy samples were taken from the right flexure of the colon and from rectum in 12 patients with active and 7 with inactive colonic CD, 33 patients with active and 24 with inactive UC, and from 11 control subjects. Cell suspensions were prepared, and analysed by flow cytometry. Eosinophils with a high surface expression of CD44 were classified as activated. Staining with fluorescein di-acetate (FDA), propidium iodide (PI) and annexin V was used to evaluate cell survival / apoptosis. We also assessed the impact of Th1 and Th2 cytokines on CD44 expression.

Results: Eosinophil activity was increased during active CD and UC compared to controls with no difference between the two patient groups. In contrast, we found significant differences in eosinophil activity between the groups in quiescent disease: whereas the activity increased substantially during inactive UC, it tended to decrease during inactive CD. CD44 expression on peripheral blood eosinophils increased by incubation with IL-5, but not with IL-13 or IFN-γ. The survival of intestinal eosinophils was increased in inflamed tissue compared to in healthy tissue in both diseases. Fewer PI-positive eosinophils were found in UC than in CD comparing non-inflamed tissue.

Conclusions: We observed different patterns of eosinophil activation in CD and UC, with the highest activity during quiescent UC, and the lowest during quiescent CD. The diverse mechanisms of eosinophil activation in CD and UC may be due to different cytokine milieus, a notion that is supported by our experiments with recombinant Th1 and Th2 cytokines. Eosinophil survival in inflamed intestinal tissue is significantly increased in both diseases.

This project was funded by a grant from The Swedish Research Council.

POSTER DISCUSSION SESSION II

POSTER #46

CHANGES IN EOSINOPHIL FUNCTION IN AGING ASTHMA SUBJECTS

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Background: Allergic asthma is underdiagnosed and undertreated in the aging population. In addition, as the US population ages we will see an increase in the number of asthma cases in the aging population. It is not known if this population has unique features of asthma which may require a different approach to diagnosis and management.

Eosinophils are an important inflammatory cell in the pathogenesis of asthma. They can contribute to inflammation and tissue injury in the airway by the release of toxic granules proteins, reactive oxygen species, cytokines, and lipid mediators. Whether there are any changes in these eosinophil functions with age that affect asthma is not known.

Objectives: To determine whether the presence of eosinophils or their functional activities differ between aging and young asthma subjects.

Methods: Human subjects with mild to moderate asthma between the ages of either 20-40 (younger) and 55-80 (aging) were recruited. Baseline characterization of lung function, sputum analysis and isolation of peripheral blood eosinophils were performed. Purified eosinophils were tested for several functional activities, specifically, IL-5 stimulated eosinophil derived neurotoxin (EDN) production, and phorbol myristic acid (PMA) stimulated superoxide anion production. The sputum was analyzed for degranulation of eosinophils by measuring EDN levels.

Results: Asthma characterization was comparable in the two age groups. Analysis of cell distributions in the sputum revealed no difference in the percentage of eosinophils between both groups. However, IL-5 stimulated EDN degranulation in vitro was significantly diminished in eosinophils from aging asthma subjects ($p=0.025$) compared to young asthma subjects. In addition, a trend towards diminished eosinophil peroxidase activity in PMA stimulated eosinophils of aging asthma subjects was found ($p=0.095$). Furthermore, higher EDN levels in the sputum from aging asthma subjects was found ($p=0.199$).

Conclusions: At baseline, the presence of eosinophils is comparable in the airway of aging and young asthma subjects. However, despite our finding that IL-5 stimulated EDN degranulation in vitro was significantly diminished in aging asthma subjects, we found an equivalent if not greater level of EDN in the airway of aging asthma subjects.

This project was funded by a grant from T. Franklin Williams Scholar Program, co-sponsored by Atlantic Philanthropies, John A. Hartford Foundation, American Academy of Allergy, Asthma, and Immunology, Association of Subspecialty Professors and UW Clinical Research Scholar Award (NIH K12, RR01761401)

POSTER DISCUSSION SESSION II

POSTER #47

EXPRESSION OF SIALOSIDE LIGANDS FOR SIGLEC-8 IN HUMAN LUNG AND NASAL POLYPS

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Background: Sialic acid-binding immunoglobulin-like lectin-8 (Siglec-8) is an inhibitory surface receptor expressed on human eosinophils, basophils and mast cells. Siglec-8 recognizes the carbohydrate structure NeuAcα2–3Galβ1–4[Fucα1–3](6-O-sulfo)GlcNAc, also referred to as 6'-su-sLe^x, with high-affinity, but the binding characteristics and tissue distribution of the natural ligands are unknown. Interaction of Siglec-8 with its natural ligands might be an important means to control allergic inflammation.

Objectives: To explore the tissue expression of the Siglec-8 ligand and the unique sialyltransferases and sulfotransferase required for the biosynthesis of 6'-su-sLeX in human tissues from the upper and lower airways.

Methods: Histochemical studies were performed to assess the distribution of ligands on selected human tissues using Siglec-8-Ig fusion proteins and Ig controls. The sialic acid binding dependency of Siglec-8-Ig fusion protein was confirmed by pre-treating tissue sections with neuraminidase. Staining patterns of Siglec-8-Ig protein were compared to patterns of plant lectins specific for α2,3 or α2,6-linked sialic acids (Maakia amurensis, [MAA] and Sambucus nigra, [SNA], respectively). Taqman PCR was used to detect α2,3-sialyltransferase (ST3Gal IV) and keratin sulfate galactose 6-O sulfotransferase (KSGal6ST) in the human type II pneumocyte cell line A549. Conventional RT-PCR was used to detect KSGal6ST in tissues.

Results: Studies revealed the expression of a Siglec-8 ligand by the epithelium of small airways in the lung, as well as by type II pneumocytes. Interestingly, the tissue expression of the ligand was strongly expressed by nasal polyp epithelium. The staining pattern of Siglec-8-Ig protein was epithelial and similar to the plant lectin MAA, which like Siglec-8 binds specifically to α2-3-linked sialic acid structures, whereas staining with SNA, which preferentially binds to α2-6 linked sialic acid, was diffuse and parenchymal. Staining with both the Siglec-8-Fc protein and MAA was completely abolished after removal of sialic acid by tissue pre-treatment with neuraminidase. The expression of the enzymes ST3Gal IV and KSGal6ST, which are required for 6'-su-sLeX expression, was confirmed by real-time PCR experiments in that both enzymes were found to be constitutively expressed in A549 cells. Broader tissue screening by conventional PCR revealed expression of KSGal6ST in human lung, liver and skin, but no expression in kidney and heart.

Conclusions: Siglec-8 ligands, and enzymes for their synthesis, are expressed by epithelial cells of the upper and lower airways, as well as type II pneumocytes and perhaps other non-airway cells. Given the strong pro-apoptotic and inhibitory effects of Siglec-8, the expression of its glycan ligands on respiratory epithelium may play an important regulatory role in allergic diseases of the upper and lower airways.

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POSTER DISCUSSION SESSION II

POSTER #48

ASSOCIATION BETWEEN P-SELECTIN AND EOSINOPHIL β1 INTEGRIN ACTIVATION IN ASTHMA

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Background: β1 integrin activation state on eosinophils in blood, as assessed with activation-sensitive monoclonal antibody N29, correlated inversely with forced expiratory volume in 1 second (FEV_1) in an inhaled corticosteroid withdrawal study of mild asthmatics. We hypothesized that β1 activation is caused by the interaction of P-selectin with eosinophils, thus increasing the likelihood of eosinophil arrest and egress in the bronchial circulation.

Objectives: To learn whether P-selectin causes β1 activation of blood eosinophils and whether eosinophil-bound P-selectin is associated with activation of β1 and decreased FEV_1 in asthma.

Methods: Subjects (n = 23) with mild/moderate asthma were studied during and after resolution of an upper respiratory tract infection. FEV_1, β1 activation state, and P-selectin on eosinophils were measured. In *in vitro* experiments, soluble recombinant P-selectin was added to whole blood, and N29 epitope expression and adhesion of eosinophils to vascular cell adhesion molecule-1 (VCAM-1) under static and flow conditions were assayed.

Results: Eosinophil-associated P-selectin correlated with N29 reactivity ($r_s = 0.82$, $p < 0.0001$) and inversely with FEV_1 ($r_s = -0.59$, $p = 0.005$). In dual labeling studies, the population of blood eosinophils with high level of associated P-selectin had higher β1 activation state than the P-selectin-low population. Addition of soluble P-selectin to blood enhanced N29 reactivity, static adhesion and adhesion under flow to VCAM-1.

Conclusions: Level of P-selectin associated with circulating eosinophils correlates with β1 activation state and FEV_1. P-selectin addition to blood causes enhanced eosinophil β1 activation and adhesion to VCAM-1. These findings are compatible with a scenario in which P-selectin from activated platelets and/or endothelial cells associates with P-selectin glycoprotein ligand-1 (PSGL-1) on eosinophils, activating β1, and stimulating eosinophil arrest on VCAM-1 and subsequent egress to the airway. Therefore, P-selectin-mediated eosinophil β1 activation could potentially be a reasonable therapeutic target in asthma.

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POSTER DISCUSSION SESSION II

POSTER #49

ARE THERE DIFFERENCES IN THE INNATE REACTIVITY OF EOSINOPHILS TO AIRBORNE ALLERGENS BETWEEN ATOPIC AND NON-ATOPIC INDIVIDUALS?

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Background: The eosinophilic granulocyte is a key component of allergic disorders. One possible explanation for why this particular leukocyte is involved in allergic reactions may be gleaned from recent findings indicating that eosinophils can be directly activated by airborne allergens.

Objectives: To investigate whether eosinophilic reactivity to inhalant allergens differs between atopic and non-atopic individuals on the one hand, and whether natural exposure to allergen modulates this reactivity.

Methods: Volunteers were chosen according to IgE status measured by the Phadiatope test and occurrence of allergic symptoms and eosinophils were purified from heparinized venous blood. 32 subjects (19 atopic and 13 non-atopic) were examined during birch pollen season and 16 subjects (8 atopic and 8 non-atopic) were studied out of season. Eosinophils were stimulated with extracts from birch and grass pollen, house dust mite (HDM) and cat dander. The capacity of stimulated cells to migrate *in vitro*, release major basic protein (MBP), eosinophil peroxidase (EPO) and T_H1/T_H2 cytokines.

Results: There were no differences between atopic and non-atopic individuals regarding the magnitude of the MBP and EPO release from eosinophils stimulated *in vitro* with birch pollen extract. Eosinophils from both donor groups released low levels of IL-4 upon stimulation with allergen extract both in and out-of-season. Intriguingly, eosinophils from atopic and non-atopic individuals alike, responded with low-grade release of TNF when stimulated with birch pollen out of season, which was not seen during season. This suggests the capacity of eosinophils to produce and secrete TNF may be down regulated during the birch pollen season.

Conclusions: Our findings indicate no differences in the reactivity of eosinophils to inhalant allergens that depend on atopic status.

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POSTER DISCUSSION SESSION II

POSTER #50

IL-1 MEDIATES THE DELAYED EOSINOPHIL ACCUMULATION AROUND NERVES AND SUBSEQUENT AIRWAY HYPERREACTIVITY AFTER OZONE

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Background: Eosinophil major basic protein is an endogenous antagonist for M_2 muscarinic receptors. This is important in lungs since M_2 receptors control acetylcholine release from parasympathetic nerves. 1 day after ozone eosinophils are recruited to airway nerves, release major basic protein that blocks M_2 receptors, increasing acetylcholine release and increasing vagally induced bronchoconstriction. However, while eosinophils are still present 3 days post ozone, they have a different role in that depleting them increases ozone induced airway hyperreactivity (AJP 2005,289:L627).

Objectives: To test whether ozone induces new eosinophil production over 3 days, and whether IL-1, which is increased by ozone, mediates airway hyperreactivity and eosinophil accumulation around nerves 1 and 3 days post ozone exposure.

Methods: Guinea pigs were treated with an IL-1 receptor antagonist (anakinra, 30mg/kg i.p.) 30 minutes before ozone (2ppm, 4 hours). One or 3 days later animals were anesthetized, tracheostomized, paralyzed, and ventilated. Electrical stimulation of both vagus nerves caused bronchoconstriction that was significantly potentiated in ozone compared to air exposed guinea pigs. Peripheral blood, bronchoalveolar lavage and lungs were harvested and analyzed by immunocytochemistry. In a separate set of experiments guinea pigs were treated with 5-bromo-2'-deoxyuridine (BrdU 50mg/kg, ip) 30 minutes before ozone to track production of newly produced cells ($BrdU^+$) in bone marrow, peripheral blood, bronchoalveolar lavage, and lungs 1-3 days post ozone.

Results: The IL-1 receptor antagonist had no effect 1 day post ozone. However, it inhibited hyperreactivity and eosinophil recruitment to nerves 3 days post ozone. This is significant because BrdU experiments demonstrated that by 3 days post ozone 83% of eosinophils in the lungs were new. The IL-1 receptor antagonist also decreased deposition of major basic protein in the airways 3 days after ozone exposure.

Conclusions: These data confirm that mechanisms of ozone induced airway hyperreactivity are changed over 1-3 days after exposure and demonstrate that IL-1 is important at day 3 but not at day 1. Eosinophils participate at both time points. They are clearly detrimental at day 1, but are not controlled by IL-1. By day 3, ozone selectively recruits new eosinophils to lungs and the new eosinophils appear to have a different role since depleting them worsens hyperreactivity while inhibiting their recruitment to nerves with an IL-1 receptor antagonist is beneficial.

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POSTER DISCUSSION SESSION II

POSTER #51

ATROPINE PRETREATMENT ENHANCES EOSINOPHIL ACTIVATION IN AIRWAYS OF ANTIGEN CHALLENGED GUINEA PIGS

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Background: Eosinophil major basic protein (MBP) is an endogenous antagonist for M_2 muscarinic receptors. In the lungs of antigen challenged animals, eosinophils recruited to airway parasympathetic nerves release MBP that blocks inhibitory M_2 muscarinic receptors on airway nerves. This increases acetylcholine release, resulting in increased bronchoconstriction and airway hyperreactivity. Acutely, anticholinergics block hyperreactivity in challenged animals and reverse asthma exacerbations in man; however, they are less effective in chronic management of asthma.

Objectives: To test whether pretreatment with a nonselective muscarinic antagonist, atropine, before antigen challenge affects eosinophil accumulation, activation and development of airway hyperreactivity 24 hours following antigen challenge.

Methods: Guinea pigs were sensitized and 21 days later atropine (1mg/kg, ip) or saline was given 1h before and 6h after inhaled ovalbumin challenge. Twenty-four hours later, by which time atropine had worn off, airway reactivity to electrical stimulation of both vagi was measured, as was eosinophil recruitment to the lungs. Eosinophils from guinea pig peritoneal lavage were screened for muscarinic receptor mRNA by RT-PCR and for muscarinic receptor protein by immunocytochemistry. RNA isolated from $\geq$99% pure eosinophils was reverse transcribed into cDNA and screened by real-time PCR using primers specific to 5 subtypes of muscarinic receptors. Eosinophils were labeled with antibodies specific to muscarinic receptors and visualized with fluorophore tagged secondary antibodies.

Results: Eosinophils in bronchoalveolar lavage, within airway tissues and around airway nerves were significantly increased by antigen challenge. Atropine significantly decreased eosinophilia in challenged animals. However, loss of intact eosinophils was accompanied by deposition of extracellular eosinophil major basic protein in airways (assessed blindly), which was increased by challenge and significantly further potentiated by atropine. Antigen challenge caused airway hyperreactivity that was also significantly potentiated by atropine pretreatment and was attenuated by pretreatment with AbIL-5 to inhibit eosinophils in the lungs. RT-PCR and immunocytochemistry demonstrated that eosinophils express M_3 and M_4, but not M_1, M_2 or M_5 muscarinic receptors. Thus, atropine pretreatment increased eosinophil activation and potentiated airway hyperreactivity in an eosinophil dependent manner.

Conclusions: These data demonstrate that muscarinic receptor blockade before antigen challenge increases eosinophil activation and potentiates vagally mediated airway hyperreactivity, possibly by blocking inhibitory muscarinic receptors on eosinophils.

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POSTER DISCUSSION SESSION II

POSTER #52

SEQUENCE VARIATIONS OF THE EOSINOPHIL PROTEIN X / EOSINOPHIL DERIVED NEUROTOXIN GENE IN DIFFERENT ETHNICAL POPULATIONS

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Background: The eosinophil protein x / eosinophil derived neurotoxin (EPX/EDN) is one of the cationic proteins released upon eosinophil activation. EPX is known to paralyse Schistosoma mansoni in vitro.

Objectives: To investigate possible linkages between genetic variations in the EPX gene and heavy endemic exposure to many parasites and helminths.

Methods: The EPX gene sequence was analysed in 32 samples from a Ugandan population and 76 samples from a Swedish population. The intron polymorphism at position 405 (G>C) was further analysed by Real-time PCR using TaqMan® reagents in 217 samples from the Swedish population and 331 from the Ugandan population.

Results: DNA sequencing showed one polymorphism in the Swedish population (EPX405) and five in the Ugandan (EPX405, 416, 836, 980 and 1122.). The EPX405 C-allele frequencies was 19.8% and 5.3% for the Swedish and Ugandan material respectively, showing a significant difference ($p<0.0001$) between the populations.

Conclusions: The EPX gene is highly conserved in the Swedish population, whereas the Ugandan population had a higher content of polymorphisms, at the same time the prevalence of the EPX405 G-allele in the African materials is lower than in the Swedish population. Other studies have shown a relation between the EPX405 genotype and EPX/EDN cellular content (Jönsson et al, to be published), our results suggest a lower cellular content of EPX/EDN in the parasite-exposed population.

POSTER DISCUSSION SESSION II

POSTER #53

THE YIN YANG RELATIONSHIP BETWEEN THE CELLULAR CONTENT OF THE EOSINOPHIL CATIONIC PROTEIN AND THE EOSINOPHIL PROTEIN X / EOSINOPHIL DERIVED NEUROTOXIN IN BLOOD EOSINOPHILS IS RELATED TO THE ECP562(G>C) AND EPX405(G>C) POLYMORPHISMS

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Background: The human eosinophil granulocytes contain basic proteins located in the secondary granules. Two of these are the eosinophil cationic protein (ECP) and eosinophil protein x/eosinophil derived neurotoxin (EPX/EDN), which share a high degree of sequence homology. We found previously that the cellular content of ECP in blood eosinophils was related to the ECP562(G>C) polymorphism in the 3'UTR of the ECP gene.

Objectives: To study the genetic control of the cellular contents of ECP and EPX in blood eosinophils in health and disease.

Methods: Sequencing of the ECP and EPX genes was performed on Beckman Coulter CEQ™ 2000 and 8000 DNA analysis systems. The EPX405(G>C) genotype was specifically analyzed by Real-Time PCR using TaqMan® reagents. The eosinophil contents of ECP and EPX were determined by the extraction of whole blood and subsequent analysis of the proteins by specific radioimmuno-assays. The contents were corrected for the average contents of ECP and EPX in the extracted neutrophils.

Results: The eosinophil content of ECP was related to the ECP562(G>C) polymorphism with the lowest content related to the 562CC genotype. No other relationships to the ECP contents were found in the ECP gene. The ECP562(G>C) polymorphism also showed a relationship to the content of EPX and with the highest content related to the 562CC genotype. The EPX405(G>C) polymorphism was related to the content of ECP and the content of EPX with the highest levels of ECP and the lowest levels of EPX related to the EPX405GG genotype.

Conclusion: The cellular contents of ECP and EPX are related to the ECP562(G>C) and EPX405(G>C) genotypes in a yin yang manner. These findings may reflect the common ancestry of the two genes.

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POSTER DISCUSSION SESSION II

POSTER #54

THE T-CELL DEATH ASSOCIATED GENE-8 (TDAG8) RECEPTOR IS AN EOSINOPHIL PROTON-SENSING MOLECULE THAT CRITICALLY REGULATES AIRWAY EOSINOPHILIA.

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Background: T-cell death-associated gene-8 (TDAG8) is a novel acid-sensing G-protein coupled receptor expressed on multiple immune cell lineages that we identified as upregulated in two mouse models of allergic lung inflammation.

Objectives: We investigated the role of TDAG8 on eosinophils in vitro and in vivo in allergic airway disease.

Methods: TDAG8-deficient mice were generated by insertion of the Enhanced Green Fluorescent Protein (EGFP) gene replacing the TDAG8 gene via homologous recombination. Intracellular cAMP level was measured via ELISA in spleen-derived eosinophils from IL-5 transgenic mice. Sensitized TDAG8-deficient and wild type mice were challenged with allergen, and we obtained bronchoalveolar lavage fluid (BALF), lung tissue, blood and serum for analysis. We analyzed the viability of BALF cells using the viability dyes AnnexinV and 7AAD.

Results: We first demonstrate substantial TDAG8 promoter activity in eosinophils suggesting that the acid-sensing TDAG8 is expressed in eosinophils. Indeed, protons induced dose-dependent increase in cAMP accumulation in eosinophils (pH=5.0: 2700 +/- 130 fmol/10^7 cells and pH=7.5: 550 +/-150 fmol/10^7 cells, p=0.002). Notably, proton-induced cAMP accumulation was completely ablated in TDAG8-deficient mice indicating a non-redundant and essential role for TDAG8 as the critical acid-sensing molecule expressed by eosinophils. Given that the asthmatic lung has been shown to represent an acidic microenvironment, we hypothesized that TDAG8 would be particularly involved in an experimental model of allergic airway inflammation. Notably, there was an 80% ± 10% attenuation of antigen-induced eosinophilia in the BALF (p=0.003-0.01, n=6 experiments) even though these mice had normal eosinophil development (as assessed by peripheral blood eosinophil levels and bone marrow colony forming units [CFU-eosinophils]), systemic sensitization (as assessed by antigen-specific Ig levels) and lung Th2 cytokine levels (e.g. eotaxin-2, IL-5 and IL-13) after allergen-challenge compared to wild type mice. These data suggest that TDAG8-/- eosinophils were appropriately recruited to the lung parenchyma but their numbers are significantly reduced in the BALF. Because several published reports have demonstrated that increasing intracellular cAMP in eosinophils leads to increased survival, we hypothesized that TDAG8 maintains eosinophil survival in the allergic airway. Notably, TDAG8-/- BALF eosinophils were significantly less viable (TDAG8+/+: 82% +/- 10% and TDAG8-/-: 50% +/- 8%, p=0.01).

Conclusions: TDAG8 is a critical eosinophil acid sensing receptor that regulates antigen-induced eosinophilia likely by controlling cell survival.

POSTER DISCUSSION SESSION II

POSTER #55

ASPIRIN-INDUCED ASTHMA IN LTC4-SYNTHASE TRNSGENIC MICE

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Background: Leukotriene C4 (LTC4) have been recognized for their powerful bronchoconstricting effects. An inhibition of cyclooxygenase (COX) pathway by NSAIDs in turn activates 5-lipoxygenase (5-LO)/LTC4 synthase pathway, and it is thought to associate with development of aspirin-induced asthma (AIA).

Objectives: To investigate roles of LTC4 for development of AIA in LTC4-synthase transgenic (LTC4S-Tg) mice carrying murine LTC4 synthase cDNA under control of the CMV enhancer and chicken beta actin promoter.

Methods: (1) Saline or sulpyrine solution as a COX inhibitor were administered by inhalation to OVA-sensitized mice at 48h after the 1% OVA antigen challenge. Airway resistance (Penh) was measured by barometric whole body plethysmography at 2h after sulpyrine inhalation. Subsequently bronchial alveolar lavage (BAL) was performed (2) Granulocytes in BAL-fluids (BALF) and splenic mast cells from the mice were cultured in the presence of sulpyrine for 24h. (3) The levels of LTC4, PGD2 and PGE2 in the BALF or culture supernatants were determined by EIA. (4) We investigated effects of pretreatment with an oral administration of pranlukast, a blockade of cysteinyl LTs receptor on the sulpyrine-induced airway responses.

Results: (1) LTC4S-Tg mice showed a significant increase in Penh after sulpyrine inhalation by a dose dependent manner (1, 10, 50 mg/ml). In contrast, wild type mice showed no significant changes in Penh by sulpyrine. (2) LTC4 synthesis in BALF were augmented in LTC4S-Tg mice to a greater extent than those in wild type mice after sulpyrine inhalation, while syntheses of PGs were similarly inhibited in both mice. The augmentation for sulpyrine-induced LTC4 synthesis in the Tg mice was observed in granulocytes and mast cells as well as BALF. (3) Pretreatment of pranlukast prevented significantly the sulpyrine-induced airway obstruction in LTC4S-Tg mice.

Conclusions: We clearly indicated that activation of 5-LO/LTC4 synthase pathway plays an important role for the pathogenesis of experimental AIA in mice.

POSTER DISCUSSION SESSION II

POSTER #56

A ROLE FOR EOSINOPHILS IN INTESTINAL MAST CELL ACCUMULATION AND ALLERGEN-INDUCED DIARRHEA

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Background: In a murine model of gastrointestinal food hypersensitivity, we recently demonstrated that OVA-induced allergic diarrhea was an IgE / mast cell (MC) (and not eosinophil)-mediated process.

Objectives: We now investigate if eosinophil levels affect gastrointestinal mast cell accumulation at baseline and following oral-allergen exposures.

Methods: Wild type BALB/c mice were compared to CCR-3 deficient mice and ΔdblGATA-1 deficient mice. Mice were sensitized with OVA/alum followed by repeated intragastric administrations of 10mg of OVA or saline. Chloroacetate esterase staining for MC and major basic protein (MBP; a gift of J.Lee) immunostaining for eosinophils were done on jejunum and ileum paraffin sections and cells were quantitated in at least 20 high power fields (HPF). OVA-specific antibody titers and mouse MC protease-1 (MMCP-1) levels were assessed by ELISA.

Results: No MBP positive cells were observed in the small intestine of ΔdblGATA-1 deficient mice and no eosin-stained cells were detected in the blood. CCR-3 deficient mice had increased eosinophil blood levels (2-fold; $p<0.01$) and decreased intestinal eosinophil levels compared to wild type mice (4-fold; $p<0.01$). At baseline, mucosal MC levels were elevated in the small intestine of eosinophil-devoid mice (3.3±0.7 vs 1.0 ±0.3 MC/HPF; $p<0.001$). Additionally, intestinal MC levels in CCR3 deficient mice were significantly higher than in the two other groups(12.1±3.4 MC/HPF; $p<0.001$). Accordingly, plasma levels of the MMCP-1 were significantly increased in Δdbl GATA-1 and CCR-3 deficient mice compared to wild type mice ($p<0.001$). To determine if the increased MC accumulation observed in CCR-3 deficient mice was the result of increased MC progenitor recruitment to the small intestine, intestinal MC progenitor levels were assessed and shown to be similar between CCR-3 deficient and wild type mice. Consistent with a critical role for MC in allergic diarrhea, CCR-3 deficient mice were more susceptible to develop diarrhea than other groups. Diarrhea onset occurred also earlier in ΔdbleGATA-1 deficient mice compared to wild type mice.

Conclusions: Low intestinal eosinophil levels are associated with increased baseline mast cell levels and more severe allergen-induced diarrhea. While our results suggest that eosinophils partially protect against this model of oral antigen-induced intestinal anaphylaxis, it remains possible that the ΔdblGATA-1 and CCR-3 deficiencies affect mast cells independently of eosinophils.

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POSTER DISCUSSION SESSION II

POSTER #57

EOSINOPHILS MEDIATE ALUM IMMUNIZATION-ELICITED B CELL PRIMING

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Background: Aluminum hydroxide (alum), an aluminum compound long utilized as an adjuvant in human vaccinations, functions by ill-defined immunostimulatory mechanisms. Intraperitoneal administration of antigen-free alum to mice induces accumulation in spleens of an unknown IL-4-producing $Gr1^+$ myeloid cell population that is required for alum-elicited early B cell priming.

Objectives: We evaluated whether eosinophils, as innate $Gr1^+$, IL-4 producing cells, are necessary and sufficient to mediate the early *in vivo* alum-elicited priming of B cells.

Methods: Eosinophil-deficient ΔdblGATA BALB/c or wild-type (WT) BALB/c mice were injected i.p. with 100 μL (4.5 mg) alum. Six days after injection, single cell suspensions were prepared from spleens and bone marrows (BM) of the mice. Alum-elicited splenic $B220^+$ B cell priming was determined by calcium flux triggered by MHC II cross-linking and analyzed by a BD LSR II Flow Cytometer. $Gr1^+/IL\text{-}4^+$ double positive cells in spleens and BM were sorted by FACS, stained with Hema 3 differential staining and observed microscopally. In some experiments, $10x10^6$ eosinophils purified from spleens of IL-5 transgenic BALB/c mice were adoptively transferred via tail veins into ΔdblGATA BALB/c mice immediately after i.p. alum challenge, and B cell priming in the reconstituted mice was evaluated as described above.

Results: We demonstrate that the alum-elicited and -activated splenic myeloid cells are IL-4 expressing eosinophils that function to prime B cell responses. Eosinophils are the principal $Gr1^+$, $IL\text{-}4^+$ cells in the spleens and BM 6 days following i.p. alum administration. Alum-elicited splenic B cell priming developed in wild-type mice, was absent in eosinophil-deficient mice and could be reconstituted by eosinophil infusions into the eosinophil-deficient mice.

Conclusions: Eosinophils, leukocytes of the innate immune system that typically contain preformed cytokines, including IL-4, have novel immunomodulatory roles in the early priming of B cells elicited by the adjuvant alum. These findings provide insights into the mechanisms of alum adjuvanticity and elucidate a novel role for eosinophils in alum-mediated priming of B cell responses.

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POSTER DISCUSSION SESSION II

POSTER #58

A METHOD FOR THE IN VIVO ASSESSMENT OF EOSINOPHIL-SPECIFIC EFFECTOR FUNCTIONS: COMBINATION OF GENETIC AND ENGRAFTMENT STRATEGIES

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Background: Reductionistic strategies to define the roles of eosinophils and their effector molecules require the ability to assign activities unequivocally. Unfortunately, both in vitro and in vivo methods of characterizing eosinophils and their effector activities have several limitations. Genetic interventions in mice have allowed generation of both hyper- and hypo-eosinophilic strains. With these as a basis, we have developed a strategy that combines the elegance of targeted lineage ablation and IL-5 overexpression with adoptive progenitor cell engraftment to generate mice singularly deficient in eosinophil-derived inflammatory mediators.

Objective: To generate eosinophil-lineage knockouts in order to define in vivo eosinophil-derived effector functions.

Methods: We have crossed eosinophil-less mice (PHIL) with IL-5 over-expressing transgenic mice. Such mice then are given a low dose of whole body x-irradiation (<100 cGy) and an infusion of hematopoietic stem cells (HSC) from isogeneic donor mice with a deletion or mutation affecting the expression of the gene of interest.

Results: The host animal retains its genotype, including almost all lymphohematopoietic cells (LHC) other than eosinophils. Engraftment does result in a small proportion of peripheral LHC being derived from the donor HSC. However, donor HSC are the only source of eosinophils in this host owing to the constitutively expressed IL-5. By 3 weeks grafts stabilize; >95% of non-eosinophil lineage LHC are of host origin while 100% of the eosinophils are of donor origin. Furthermore, if donor and host are further distinguishable by Ly5.1 and Ly5.2, the composition of peripheral blood (or other suspensions) may be tracked by flow cytometry.

Conclusions: It is possible to define the eosinophil origin of effector molecules in the context of the physiologically relevant environment in vivo by constructing mice in which the only cells deficient in the effector are eosinophils.

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

POSTER DISCUSSION SESSION II

POSTER #59

CO-EXPRESSION OF IL-5 AND EOTAXIN-2 IN MICE CREATES AN EOSINOPHIL-DEPENDENT MODEL OF RESPIRATORY INFLAMMATION WITH CHARACTERISTICS OF SEVERE ASTHMA

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Mouse models of allergen provocation and/or transgenic gene expression have provided significant insights regarding the cellular, molecular, and immune responses linked to the pathologies occurring as a result of allergic respiratory inflammation. Nonetheless, the inability to replicate the eosinophil activities occurring in patients with asthma has limited their usefulness to understand the larger role(s) of eosinophils in disease pathologies. These limitations have led us to develop an allergen-naive double transgenic mouse model that expresses IL-5 systemically from mature T cells and eotaxin-2 locally from lung epithelial cells. We show that these mice develop several pulmonary pathologies representative of severe asthma, including structural remodeling events such as epithelial desquamation and mucus hypersecretion leading to airway obstruction, subepithelial fibrosis, airway smooth muscle hyperplasia, and pathophysiological changes exemplified by exacerbated methacholine-induced airway hyperresponsiveness. More importantly, and similar to human patients, the pulmonary pathologies observed are accompanied by extensive eosinophil degranulation. Genetic ablation of all eosinophils from this double transgenic model abolished the induced pulmonary pathologies, demonstrating that these pathologies are a consequence of one or more eosinophil effector functions.

POSTER DISCUSSION SESSION II

POSTER #60

ESOPHAGEAL REMODELING DEVELOPS AS A CONSEQUENCE OF TISSUE SPECIFIC IL-5-INDUCED EOSINOPHILIA IN MICE

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Background: Eosinophilic esophagitis (EE) is an increasingly recognized disease that mimics gastroesophageal reflux disease. Recently, EE has been associated with esophageal remodeling, but the mechanisms involved are poorly understood.

Objective: Examine the mechanism of the induction of esophageal remodeling in EE.

Methods: Esophageal remodeling was assessed by histologically examining the basal layer thickness and collagen accumulation on patient biopsies as well as in mouse esophageal tissue sections following the induction of experimental EE in wild type, IL-5 deficient and IL-5 transgenic mice. The semi-quantitative analysis of basal layer and lamina propria collagen thickness was measured by morphometric analysis and IL-5 gene expression by real time PCR analysis.

Results: An impressive accumulation of collagen in the epithelial mucosa and lamina propria, and basal layer thickening was observed in the esophageal biopsies from EE patients compared to normal individuals as well as in mice with experimental EE. Significantly reduced lamina propria collagen and basal layer thickness was observed in IL-5 deficient mice compared to wild type mice following the induction of experimental EE. Furthermore, the esophagus of CD2-IL-5 transgenic mice showed increased thickness of basal layer and collagen accumulation compared to non-transgenic mice, yet IL-5 intestine transgenic (iIL-5) mice did not have EE-like esophageal changes. Although, both CD2-IL-5 and iIL-5 mice show eosinophils the esophagus. Additionally, the real time PCR analysis revealed increased IL-5 levels in the esophagus of allergen challenged and CD2-IL-5 transgenic mice compared to saline challenged and iIL-5-transgenic mice.

Conclusion: These findings provide evidence that IL-5-mediated eosinophilia is essential in the induction of esophageal remodeling.

The project was funded by the grant NIH RO1DK067255 (AM)

POSTER DISCUSSION SESSION II

POSTER #61

CD34 INVOLVEMENT IN THE DEVELOPMENT OF ALLERGIC ASTHMA AND EOSINOPHIL MIGRATION

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Background: Asthma is characterized by the infiltration of hematopoietic cells into the lung tissue and broncho-alveolar space, associated with remodeling and airway hyperresponsiveness. Prominent among infiltrating hematopoietic cells are mast cells and eosinophils, which are hypothesized to directly cause tissue inflammation and damage by local release of mediators. Recent findings have demonstrated expression of the surface sialomucin CD34 on these cell types and our lab has suggested a role for CD34 as an anti-adhesin necessary for migration.

Objectives: Previously we showed that expression of CD34 is essential for efficient mast cell migration leading us to examine its role in the development of asthma, with specific focus on roles in eosinophil and mast cell migration.

Methods: $CD34^{-/-}$ and wild-type C57Bl/6 mice were sensitized and challenged with chicken ovalbumin (OVA) as has been previously reported. To assess asthma severity, airway hyperresponsiveness was tested on a Flexivent respirometer following methacholine challenge and differential hematopoietic cell counts were performed on broncho-alveolar lavage (BAL) cells. Histological tissue preparations were stained with hematoxylin/eosin and toluidine blue for evaluation of tissue inflammation and mast cells infiltration. BAL eosinophils were also stained for CD34 expression, sorted and evaluated via *in vitro* migration assays.

Results: We found that $CD34^{-/-}$ mice had far fewer infiltrating cells than Bl/6 controls and that all hematopoietic subsets were significantly reduced. Histological analysis revealed attenuation of both inflammation and mast cell counts in $CD34^{-/-}$ mice and similarly, airway hyperresponsiveness in $CD34^{-/-}$ OVA-challenged mice was lower and comparable to that of unsensitized Bl/6 mice. Interestingly, BAL and tissue-derived eosinophils in Bl/6 mice were found to express significant levels CD34 and we noted a 57.2% reduction in the ability of $CD34^{-/-}$ eosinophils to migrate towards eotaxin *in vitro* compared to Bl/6 eosinophils. Follow-up studies indicate higher blood and lowered lung tissue eosinophil numbers in the $CD34^{-/-}$ OVA-challenged mice, supporting our model that CD34 is required for efficient migration from the peripheral blood into diseased tissue.

Conclusions: Our findings suggest that CD34 expression on mast cells and eosinophils is required for efficient migration of these cells types into the lung tissue and alveoli in the asthma response. As a result of decreased infiltration, the disease course is lessened, suggesting a potential therapeutic avenue for new treatments targeting CD34 expression.

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POSTER DISCUSSION SESSION II

POSTER #62

ALLERGEN CHALLENGE INDUCES A GENOME-WIDE TH1 TRANSCRIPTOME SIGNATURE

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Background: Current paradigm suggests that allergic airway inflammation is mediated by the unopposed production of Th2 cytokines and pro-inflammatory chemokines.

Objectives: Our objective was to test the paradigm that RWE challenge induces a Th2 predominant genomic signature.

Methods: To test the accuracy of this paradigm, we performed high-throughput genearray analyses and quantitative PCR (qPCR) on lung RNA in mice sensitized and challenged with ragweed extract (RWE).

Results: RWE challenge upregulated Th2 associated genes IL4, IL5 Ccl2 and Ccl7 in the lungs by 4 hrs. Unexpectedly, RWE challenge simultaneously induced Iigp, Tgtp, Gbp1, Cxcl9, Cxcl10, Socs1 and Gadd45g in the lungs, genes that have previously been described as IFN-g or Th1 dependent. Augmentation of local Th1 milieu by co-administration of IL12 or CpG further upregulated these genes. Abolition of the Th1 response by disrupting IFN-g gene abrogated RWE challenge induced upregulation of Iigp, Tgtp, Gbp1, Cxcl9, Cxcl10 and Socs1 but not Gadd45g. Disruption of IFN-g also augmented airway eosinophil recruitment and airway hyperresponsiveness (AHR).

Conclusion: We propose that the Th2 and pro-inflammatory chemokine response induced by allergen challenge is counterbalanced by a rapid IFN-g dependent Th1 transcriptome signature characterized by upregulation of Iigp, Tgtp, Gbp1, Cxcl9, Cxcl10 and Socs1. Absence of this Th1 signature is associated with worsening of Th2-mediated allergic airway inflammation and AHR.

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POSTER DISCUSSION SESSION II

POSTER #63

ANTI-INFLAMMATORY EFFECTS OF *LAFOENSIA PACARI* AND ELLAGIC ACID IN A MURINE MODEL OF ASTHMA

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Background: We have shown that the ethanolic extract of *Lafoensia pacari* inhibits eosinophilic inflammation induced by *Toxocara canis* infection, and that ellagic acid is the secondary metabolite responsible for the anti-eosinophilic activity seen in a model of β-glucan peritonitis.

Objectives: In the present study, we investigated the preventive and curative effects of *L. pacari* extract and ellagic acid on allergic lung inflammation using a murine model of asthma.

Methods: Female BALB/c mice, 15-20 g, were immunised on days 0 and 7 by s.c. injection of 4 μg of ovalbumin (OVA) and 1.6 mg of aluminium hydroxide in 0.4 ml of saline followed by two intranasal challenges (days 14 and 21) with 10 μg of OVA in 50 μL of saline. To study preventive anti-inflammatory effects, *L. pacari* (200 mg/kg), ellagic acid (10 mg/kg), or vehicle (water) were administered orally to each group of mice for 22 days, starting from the day of immunisation. To study the curative effects mice received oral treatments of *L. pacari* (200 mg/kg), ellagic acid (0.1, 1, or 10 mg/kg) or vehicle (water) daily from day 18 to day 22. All determinations were made at 24 h after the second ovalbumin challenge. The leukocyte counts and cytokine levels were performed in the bronchoalveolar lavage fluid (BALF). To determine airway responsiveness (AHR) mice were placed in a single whole-body plethysmographic chamber, where respiratory parameters were measured after an aerosol of methacholine. The cysteinyl-leukotriene levels in the lung homogenates by were performed by specific enzyme immunoassay.

Results: In BALF, preventive treatment with *L. pacari* and ellagic acid inhibited neutrophil and eosinophil counts. *L. pacari* reduced IL-4 and IL-13 levels, whereas ellagic acid reduced IL-4 and IL-5 levels. Curative treatment with *L. pacari* and the highest ellagic acid dose reduced neutrophil and eosinophil numbers, also inhibiting IL-4, IL-5, and IL-13. Neither *L. pacari* nor ellagic acid reduced inhibited the AHR and cysteinyl leukotriene synthesis in the curative treatment.

Conclusions: *L. pacari* and ellagic acid are effective eosinophilic inflammation suppressors, suggesting a potential for treating allergies.

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POSTER DISCUSSION SESSION II

POSTER #64

REGULATION OF CARCINOGENESIS BY INTERLEUKIN-5 AND CCL-11: A POTENTIAL ROLE FOR EOSINOPHILS IN TUMOR IMMUNE SURVEILLANCE

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Background: One of the most enigmatic cells in the immune system is the eosinophil. Previous reports from our laboratory and other groups have suggested that eosinophils, when recruited into established tumors, exhibit potent anti-tumor activity.

Objectives: In this study we provide evidence, for the first time, which suggests that eosinophils play an important role in tumor immune surveillance.

Methods: Briefly, mice were injected subcutaneously with the carcinogen methylcholanthrene (MCA), with tumor establishment and growth monitored over a 6 month period, in WT, eosinophil-recruitment impaired CCL-11 (eotaxin-1) $^{-/-}$, eosinophil-high interleukin (IL)-5 transgenic (tg) mice, and the eosinophil-deficient IL-5/CCL11$^{-/-}$ and ΔdblGATA strains in the BALB/c background. Upon sacrifice, histological examination allowed the differentiation of the leukocyte influx into tumors and surrounding tissue. *In-vitro* analysis of eosinophil-mediated killing of fibrosarcoma cells was also performed.

Results: Genetically modified mice that have enhanced levels of circulating eosinophils are extremely resistant to tumor induction by MCA, with tumors that do arise having a "dormant" tumor phenotype. In contrast, mice that are deficient in CCL-11, and hence eosinophil trafficking deficient, are more susceptible to tumor induction. The eosinophil – deficient IL-5/CCL11$^{-/-}$ and ΔdblGATA strains were highly susceptible to tumor induction at high and low concentrations of MCA in the total absence of eosinophils. Overall, there is a strong correlation between the eosinophil content of tumors, their growth rate and frequency of appearance. Subsequent *in-vitro* analysis confirmed that eosinophils directly induce the killing of MCA-induced fibrosaromas.

Conclusions: Collectively this study establishes an important role for eosinophils in tumor immune surveillance, and further work will focus on clarifying the innate or adaptive nature of this response.

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