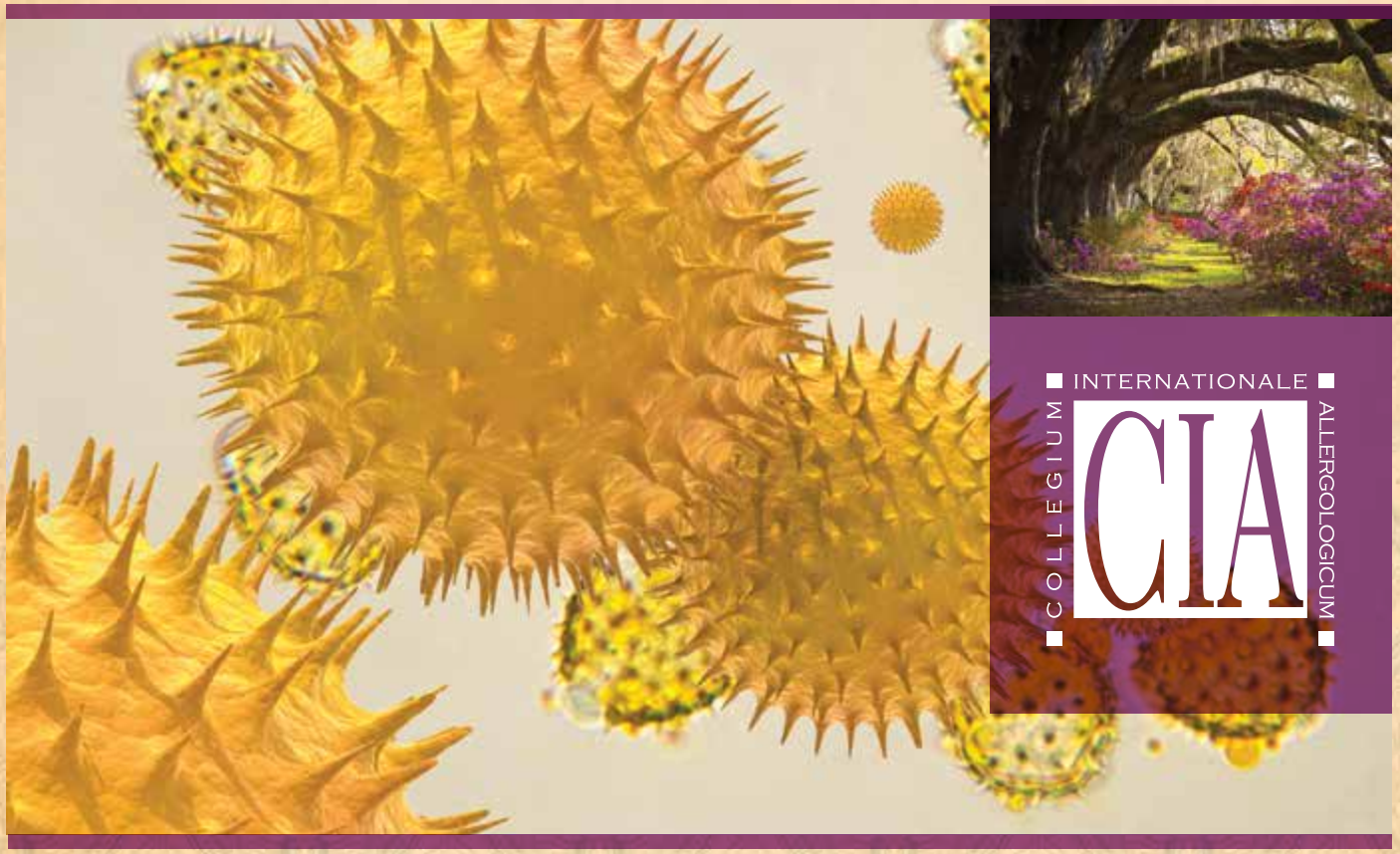
31st Symposium of the
Collegium Internationale Allergologicum
3–8 APRIL 2016
Charleston, South Carolina

TOWARDS PRECISION DIAGNOSIS AND TARGETED INTERVENTION IN ALLERGIC DISEASE



Final Program

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Dear Colleagues,

On behalf of the Council, Peter Creticos, Bruce Bochner and I, welcome to the 31st Symposium of the Collegium Internationale Allergologicum, entitled ***Towards Precision Diagnosis and Targeted Intervention in Allergic Disease***, in **Charleston, South Carolina** from **3–8 April 2016**.

From the beginning, the *Collegium* has been an exclusive club of friends pursuing excellence across the full spectrum of research in allergic diseases and allied disorders, in a spirit of open intellectual exchange, at meetings held in interesting and stimulating locations. We will be fulfilling this vision, this time in the historic and beautiful city of Charleston, founded in 1670 as Charles Towne in honor of King Charles II of England, and known for its rich history and well-preserved architecture. Charleston is widely regarded as "America's Most Friendly City" and in the past several years has been ranked as either the #1 or the #2 city in the world to visit.

Through oral and poster presentations, this Symposium will highlight the significant scientific progress being made by our discipline worldwide, including some of the latest advances in allergy, asthma and immunology. With personalized or precision medicine becoming the new direction of travel for delivering disease prevention and treatment, we have titled our Symposium to capture this futuristic endeavor.

Along with its rich history, Charleston is renowned for its outstanding plantation gardens festooned with azaleas and camellias that will be at the peak of bloom in early April. Our boat trip will take us throughout the picturesque Charleston Harbor with its strategically placed forts, lovely waterfront homes situated along the high-walled battery, and bottlenose dolphin that frequently play alongside the boats sailing in the harbor.

From the Council and the local organizers we thank you for attending the 31st Symposium look forward to seeing you over the next week in Charleston!

Kindest Regards,

Stephen T. Holgate, MD,
DSc FMedSci
CIA President

Peter S. Creticos, MD
Organizer, 31st Symposium

Bruce S. Bochner, MD
Organizer, 31st Symposium

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State of South Carolina
Office of the Governor

1205 Pendleton Street
Columbia 29201

Nikki R. Haley
Governor

Dear Friends,

On behalf of the State of South Carolina, I am pleased to welcome you to the historic city of Charleston for the 31st Symposium of the Collegium Internationale Allergologicum.

From the beginning of my administration, I have been driven to create an impact in the business community by bringing growth and innovation to the Palmetto State. Charleston and the State of South Carolina have been at the forefront of economic growth and development in the southeast region of the United States.

We have made great strides in fostering an environment in which industry, government, and academia have flourished. The state is recognized for its innovative technical education system, academic centers of higher learning – including the College of Charleston and the Medical University of South Carolina – and major corporate entities.

South Carolina has an internationally-renowned state technical college system that is dedicated to furthering workforce development. We are also home to several public and private universities that stay competitive through research, development, and innovation.

Michael and I thank you for participating in this year's Symposium and wish you all the best for a successful meeting and wonderful stay in Charleston. We hope your travels are safe and pleasant and that you have a chance to visit again soon. God bless.

My very best,

Nikki R. Haley

General Information

The 31st Symposium of the *Collegium* will be held in Charleston, South Carolina, USA. Founded in 1670 as Charles Towne in honor of King Charles II of England, it is known for its rich history and well-preserved architecture. Charleston is widely regarded as "America's Most Friendly City" and in 2014 was listed as the #2 city in the world to visit, second only to Florence, Italy. Charleston is easily accessible to other tourist destinations, including Charlotte, NC, Atlanta, GA, Orlando, FL, Boston, MA, Washington DC, and New York, NY, with non-stop flights available to each.

Currency

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

Electricity

The standard electrical voltage in the United States is 110 Volts and 60 Hertz.

Language

The official language of the 31st Symposium is English.

"Life in Science" Breakfast Discussions

These sessions are geared towards young scientists at the beginning of their career. Sessions will be in an informal session where eminent scientists will share with young investigators some of what they have experienced and learned in their "Life in Science."

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

Breaks / Lunches

Will be held in hallway outside of Carolina Ballroom at Francis Marion Hotel.

Social Events

All Social Events are included in the registration fee for both delegates and accompanying persons.

Welcome Reception

Sunday, 3 April 2016, 18:30 – 21:30
The Welcome Reception will be held at the Francis Marion Hotel with refreshments and an assortment of hors d'oeuvres.

Boat Ride

Tuesday, 5 April 2016, 16:30 - 19:00
Following in the tradition of past *Collegium* meetings, a boat ride will take place on the third day of the meeting. Bus will depart at 15:45 from the Lobby of Francis Marion Hotel.

Informal Dinner

Tuesday, 5 April 2016, 19:00 – 22:00
After the boat ride, the Informal Dinner will take place at the South Carolina Aquarium.

Gala Dinner

Thursday, 7 April 2016, 18:15 – 22:30
An elegant dinner will be held on the last evening of the Symposium at Middleton Plantation. Bus will depart at 17:30 from the Lobby of the Francis Marion Hotel.

Time Zone

Charleston is in the Eastern Time Zone (EDT), which is four hours behind the Coordinated Universal Time (UTC) and Greenwich Mean Time (GMT).

Tipping

Restaurants in Charleston generally expect a tip between 10% and 20%, as do local taxi drivers. In bars, tipping at least $1 per drink is customary. Hotel porters that assist guests with bags expect between $1-$3, depending on how many bags there are.

Travel Arrangements and Airport Transfers

The Charleston International Airport (CHS) is the closest airport to the Francis Marion Hotel.

Ground transportation will be provided on a limited basis from the airport to the Francis Marion Hotel on 3 April 2016 and 8 April 2016 from the Francis Marion Hotel to the Charleston International Airport.

On 3 April 2016 the transportation will leave the Airport to the Hotel at 9:00 AM and will run until 19:00 PM.

On 8 April 2016 the transportation will leave the Hotel to the airport at 5:00 AM and will run until 16:00 PM.

The travel time from the airport to the hotel and hotel to the airport is roughly 30 minutes so the shuttle will be running about one trip every hour.

Venue

The 31st Symposium of the *Collegium* will be held at the Francis Marion Hotel.

Weather

The average temperature in April is between 76°F/24°C and 56°F/13°C.

Optional Excursions

Grand Houses of Charleston Walking Tour

Tour two of the finest houses in the city of Charleston. The Heyward-Washington House built in 1772 houses one of the two most outstanding collections of Charleston made furniture in the city. In this lovely Georgian House President George Washington resided during his week visit to Charleston in 1791. This house also boasts one of the few original kitchen houses in the city. The kitchen house is furnished as it would have been in the late 1700's. Our second stop will be at the Nathaniel Russell House which houses an equally fine collection of Charleston made furniture. Its spiraling cantilevered staircase is truly a work of art as is the house itself. Not only will you visit these houses but along the way your guides will point out numerous architecturally significant homes and exquisite gardens.

Duration: 3-3.5 Hours with Round Trip Transfer

Date: Monday, 4 April 2016 9:00 AM – 12:00 PM

Historic Carriage Tour

Enjoy the sights of Charleston the way our ancestors did, aboard a horse-drawn carriage. A leisurely ride through narrow streets and alleyways carries you past carefully restored 18th and 19th century homes and buildings. Your knowledgeable guide relates three hundred years of history to the accompaniment of horse's hooves clopping along the pavement. The scent of tea olive from nearby gardens lingers in the air as you snap that special photograph capturing a bit of Charleston's history.

Duration: 1 Hour Carriage with Round Trip Transfer

Date: Monday, 4 April 2016 15:00 PM – 16:00 PM &
Thursday, 7 April 2016 10:00 AM – 11:00 AM

Charleston Tea Plantation

As you cross beautiful marshlands and travel lovely live oak canopied roads you will be treated to the history of the large plantations which once occupied these islands and stories of the unique culture and language of the sea island black people who worked these plantations. Included in the tour will be views of rich farm land and forests, St. John's Episcopal Church and the ***Angel Oak***, a 1400 year old live oak tree. The highlight of the tour will be a stop at the Charleston Tea Plantation, the only tea plantation in the United States. As you enter the plantation you will see thousands of tea plants growing on acres and acres of rich and fertile low country soil. A stroll under the avenue of oaks will take you to the porch of the new factory which affords remarkable views of the plantation. Begin your visit with a tour of the tea fields followed by an informative and educational tour of the factory and a visit to the gift shop stocked with interesting and exciting items including the American Classic Tea which is produced here and has a wonderful flavor.

Duration: 3.5 Hours with Transportation

Date: Tuesday, 5 April 2016 9:00 AM – 12:00 PM

Drayton Hall Plantation Tour

This tour winds down live oak canopied roads to Drayton Hall, c. 1738 considered the finest Georgian Palladian Plantation house in the south. The house has stood through the rise and fall of plantation society, survived devastating natural disaster, and remained intact in the face of modernization. Its amazing architecture and craftsmanship tell the story of a family and its community over two centuries. Your interpreter will bring this marvelous house and its family to life for you.

Duration: 4 Hours with Transportation

Date: Wednesday, 6 April 2016 9:00 AM – 13:00 PM

Fort Sumter Tour

Visit the place where the American Civil War began in 1861. Begin your tour with a cruise through Charleston's historic harbor where you can catch breathtaking views of the city and watch sea gulls and dolphins play in the harbor. Upon arrival at Fort Sumter, rangers from the National Park Service will give you an informative introduction before you tour this famous bastion and its marvelous museum.

Duration: 4 Hours with Round Trip Transfer

Date: Thursday, 7 April 2016 9:00 AM – 13:00 PM

Alain L. de Weck Travel Grants

For the third time, Alain L. de Weck Travel Grants have been awarded to young scientists that are presenting an abstract during the meeting. Each applicant was asked to provide a short letter of application, a copy of their abstract, a letter of recommendation from a current member of the *Collegium* and a copy of their Curriculum Vitae. Waived registration and a travel stipend were awarded to the following attendees:

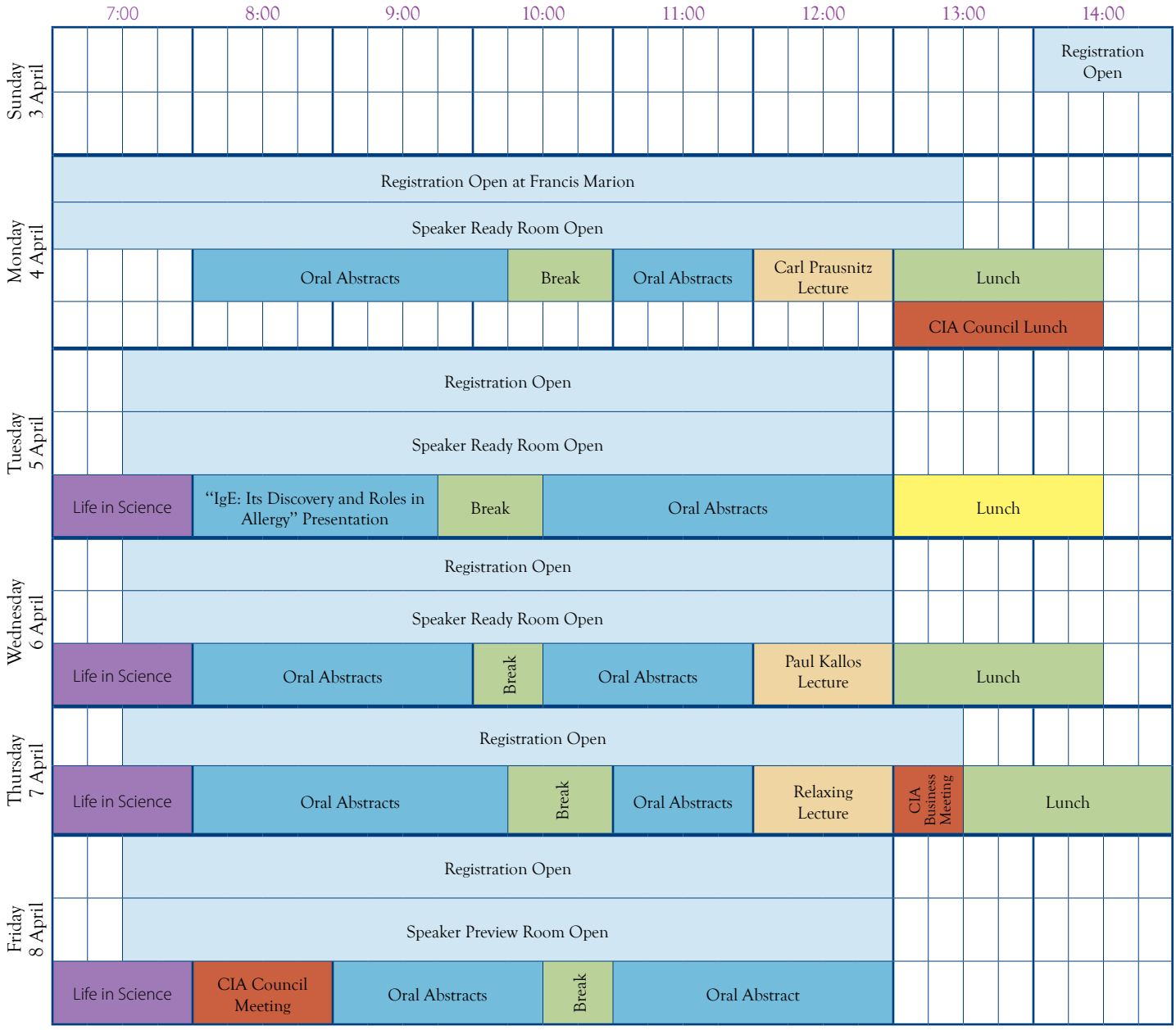
Elizabeth R. Davies, United Kingdom
Sebastian Huth, Germany
Yasutaka Mitamura, Japan
Damian Tworek, Canada
Masaru Uchida, Japan
Willem van de Veen, Switzerland
Manali Mukherjee, Canada
JJ Smit, Netherlands
Moyar Qing Ge, United States
Ulrich Zissler, Germany
Mohamed Shamji, United Kingdom
Rocio T. Martinez-Nunez, United Kingdom

Travel Grant Recipients will be awarded with a certificate during the Gala Dinner on 7 April 2016.

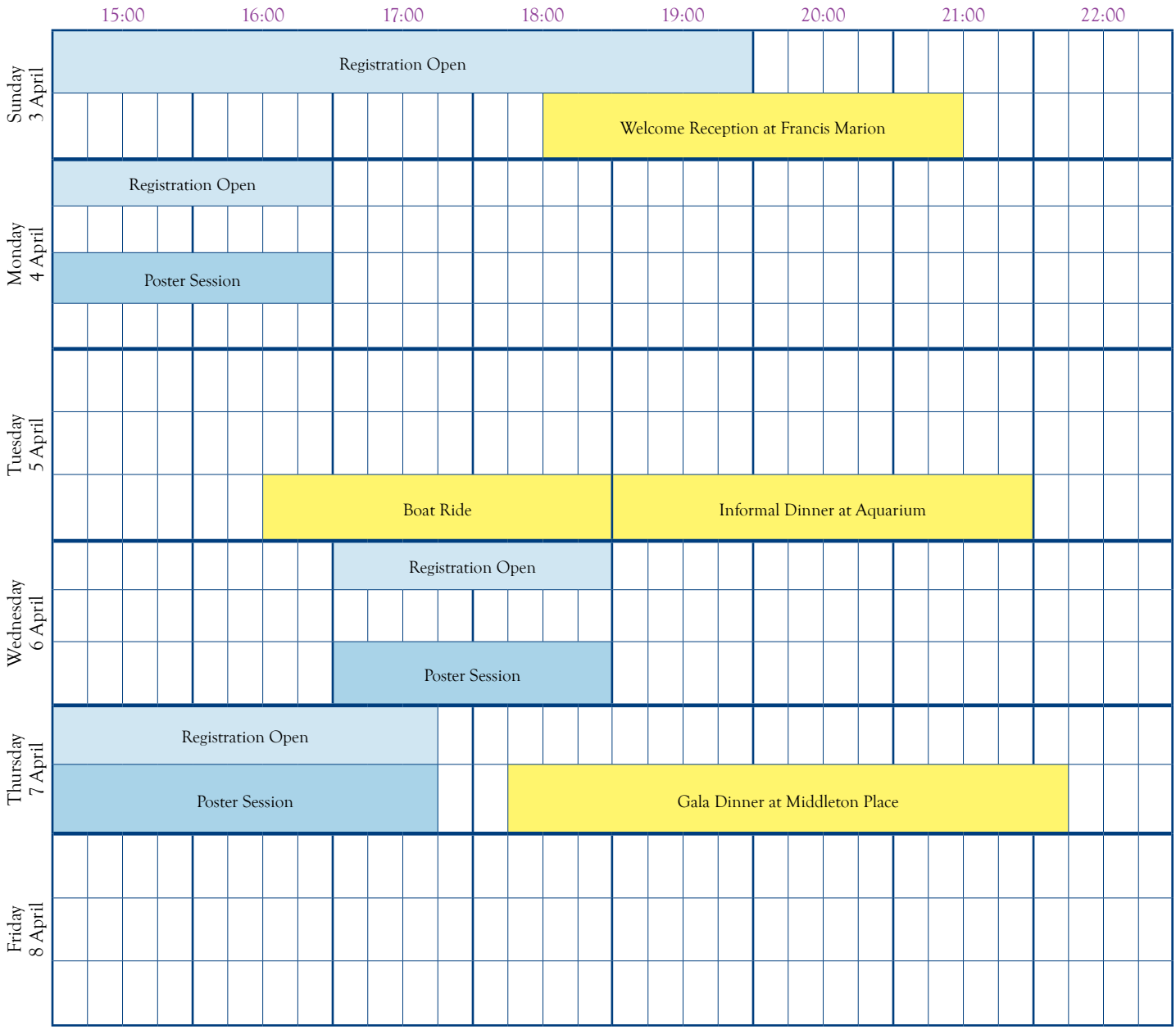
The *Collegium* would like to thank the members who contributed to the Alain L. de Weck Travel Grant Fund (included in the membership renewal form) and the following companies:

INDOOR biotechnologies

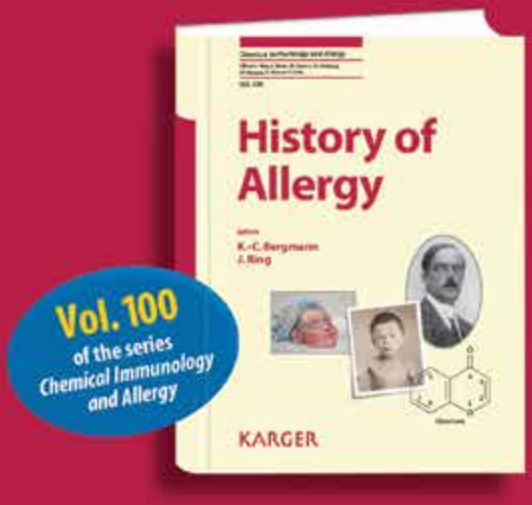
2016 Program-at-a-Glance



2016 Program-at-a-Glance



*Schedule subject to change.



A treasure trove of fascinating and richly illustrated information

History of Allergy

Editors
Karl-Christian Bergmann
Johannes Ring

This book presents a detailed and varied historical overview of the field of allergology. Beginning with insights into allergy from antiquity to the 20th century, it compiles historical reflections on the understanding of the most common allergic diseases. Important milestones in the discovery of mechanisms of allergy are described, followed by historical accounts of the detection of allergens such as pollen, dust mites, peanuts and latex, and of environmental influences such as pollution and the relationship between farmers and their environment. Particular highlights of this book are the personal reflections of and interviews with a number of pioneers of allergy, including F. Austen, J. Bienenstock, K. Blaser, A. de Weck, A.W. Frankland, K. Ishizaka, and many more.

History of Allergy
(Chemical Immunology and Allergy, Vol. 100)
Editors: Bergmann, K.-C. (Berlin); Ring, J. (Munich)
X + 426 p., 257 fig., 127 in color, 20 tab., 2014
CHF 115.00 / EUR 96.00 / USD 135.00 (hard cover)
Prices subject to change
EUR price for Germany, USD price for USA and Latin America only
ISBN 978–3–318–02194–3 (hard cover)
e-ISBN 978–3–318–02195–0

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Contents

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Map of Charleston

Schedule of Events

Sunday, 3 April 2016

14:00 – 20:00 **Registration Opens** . . . . . Upper Lobby

18:30 – 21:30 **Welcome Reception** . . . . . Colonial Room

Stephen T. Holgate, United Kingdom
Peter S. Creticos, United States
Bruce S. Bochner, United States

Monday, 4 April 2016

7:00 – 13:30 **Registration Open** . . . . . Registration Desk, Mezzanine Level

7:00 – 13:30 **Speaker Ready Room Open** . . . . . Laurens Room

7:00 – 8:00 **Poster Abstract Setup** . . . . . Gold Ballroom, Calhoun Room

8:00 – 10:15 **Oral Abstract Session 1** . . . . . Carolina Ballroom

The Origins and Activation Mechanisms of Mast Cells

Chairpersons: Gunnar Nilsson, Sweden
Francesca Levi-Schaffer, Israel

8:00 **1** ***Progenitors highly committed to the mast cell lineage identified in human blood***
Jenny Hallgren Martinsson, Sweden

8:17 **2** ***Rab12 regulates retrograde transport of mast cell secretory granules by interacting with the RILP-dynein complex***
Ronit Sagi-Eisneberg, Israel

8:34 **3** ***Interleukin-33 induces phenotypic changes in human skin-derived mast cells***
Torsten Zuberbier, Germany

8:51 **4** ***Lipid mediator metabolic profiling after IgE-induced activation of two distinct mast cell populations***
Gunnar Nilsson, Sweden

9:08 **5** ***IL-6 promotes an increase in human mast cell number and reactivity through suppression of SOCS3***
Dean D. Metcalfe, United States

9:25 **6** ***Regulation of mast cell function by the association of mitochondrial pyruvate dehydrogenase and microphthalmia transcription factor***
Ehud Razin, Israel

9:42 **7** ***Mechanism of high-potency inactivation and active-site titration of mast cell beta-tryptase by Nafamostat***
George H. Caughey, United States

9:59 **8** ***IgE-independent human mast cell activation by wheat amylase trypsin inhibitors***
Stephan C. Bischoff, Germany

10:15 – 11:00 **Break** . . . . .

11:00 – 12:00 **Oral Abstract Session 2** . . . . . Carolina Ballroom

Genetic and Environmental Factors in Allergic Disorders

Chairpersons: Kent T. HayGlass, Canada
Marianne van Hage, Sweden

11:00 **9** ***The value of whole genome sequencing in identifying genetic determinants of asthma in populations of African ancestry***
Kathleen C. Barnes, United States

11:17 **10** ***Association of season of birth with DNA methylation and allergic disease***
John W. Holloway, United Kingdom

11:34 **11** ***Environmental protection from asthma and allergy: From humans to mice and back***
Donata Vercelli, United States

Monday, 4 April 2016, *continued*

11:51 – 13:00 **Carl Prausnitz Lecture** . . . . . Carolina Ballroom

"Development and Character of Innate and Induced Mast Cells in situ: Why Does Evolution Allow for Two Subclasses of Mast Cells?"

Dr. K. Frank Austen attended Amherst College and Harvard Medical School and served his house staff training years at the Massachusetts General Hospital (MGH). He established an independent laboratory at the MGH in 1962 and moved to the Robert B. Brigham in 1966 to establish a Department of Rheumatology and Immunology which evolved into a department of the Brigham and Women's Hospital (BWH). After chairing this Department for 25 years, he shifted to the AstraZeneca Professorship of Respiratory and Inflammatory Diseases with an appointment as Director of Inflammation & Allergic Diseases Research Section. Austen has pioneered many aspects of innate immunity/inflammation through an in depth focus on the functions and regulation of arachidonic acid metabolism to the cysteinyl leukotrienes (cysLTs), the pathways for the development and phenotypic diversity of mast cells, and the pattern recognition path for activation of the alternative complement activating pathway which also serves to amplify the classical complement pathway. Austen was elected to the National Academy of Sciences and to the American Academy of Arts and Sciences in 1974 and as a foreign member of the Royal Society (UK) in 2004. Dr. Austen is the recipient of numerous medical awards including election to the American Society of Clinical Investigation, the American Academy of Arts and Sciences, the National Academy of Sciences (USA), the Royal Society (UK), and the Association of American Physicians, which chose him as the recipient of the prestigious George M. Kober Medal. Dr. Austen served as President of the American Association of Immunologist, the American Academy of Allergy Asthma and Immunology, and the American Association of Physicians. He has received honorary doctorates from the University of Paris, Hofstra University, Akron University and Amherst College.

13:00 – 14:30 **Lunch** . . . . . Colonial Room

13:00 – 14:30 **CIA Council Lunch** . . . . . Pinckney Room

14:30 – 17:00 **Poster Abstract Session** . . . . . Gold Ballroom, Calhoun Room

Genetic and Environmental Factors, Effector and Immunoregulatory Cells in Allergy and Asthma, Dendritic Cells, Mast Cells, Monocytes and Granulocytes, Lymphocytes and Mediators of Immunoregulation

Chairpersons: Kathleen C. Barnes, United States
Donata Vercelli, United States
Mariana Castells, United States
Yoseph A. Mekori, Isreal

Genetic and Environmental Factors, Effector and Immunoregulatory Cells in Allergy and Asthma

12 ***Maternal status in human pregnancy: Inherently inflammatory or anti-inflammatory?***
Kent T. HayGlass, Canada

13 ***Specific peanut allergen levels in foods: Dosing associated with oral immunotherapy and with the prevention of peanut allergy.***
Martin D. Chapman, United States

14 ***IgA responses to the gut microbiota in infants in relation to allergy development***
Maria Christina Jenmalm, Sweden

15 ***The role of early life risk factors for food, seasonal and perennial allergy in puberty.***
Karin C. Lodrup Carlsen, Norway

18 ***Genetic polymorphisms of antioxidant enzymes and maculopapular eruption caused by anti-tuberculosis drugs***
Ho Joon Yoon, Korea

19 ***Association of single nucleotide polymorphisms of protease-activated receptor-2 (Coagulation Factor II (Thrombin) Receptor-Like 1; F2RL1) gene with asthma***
Harissios Vliagoftis, Canada

20 ***Genetic variation influences the nasal microbiome***
Jayant Pinto, United States

Monday, 4 April 2016, *continued*

21 ***Association of circadian gene activation with expression and function of the glucocorticoid receptor in lung epithelial cells***
Angela Haczku, United States

22 ***Risk factors for COPD overlap in late-onset elderly asthma***
You-Young Kim, Korea

Dendritic Cells, Mast Cells, Monocytes and Granulocytes

23 ***Type I and type II interferons or viral activation induce selective human mast cell production of IL-1R antagonist and VEGF-A***
Jean S. Marshall, Canada

24 ***Mast cells are critical for the healing of infected wounds in mice***
Marcus Maurer, Germany

25 ***Elimination of human lung mast cells in situ by the anti-malarial drug mefloquine: a new principle for targeting mast cells in asthma?***
Gunnar Pejler, Sweden

26 ***Combining micro-computed tomography and immunohistochemistry to visualise mast cell 3D distribution in Idiopathic Pulmonary Fibrosis***
Jane A. Warner, United Kingdom

27 ***Papain activates human mast cells to release pro-inflammatory mediators via its enzymatic activity***
Francesca Levi-Schaffer, Israel

28 ***Statins suppress mast cell function and survival***
John J. Ryan, United States

29 ***Mechanisms of eosinophil degranulation and impact on airway responses in allergic asthma***
Paige Lacy, Canada

30 ***Morphological grading of bone marrow biopsy for the diagnosis of mast cell activation syndrome.***
Cem Akin, United States

31 ***Prognostic and predictive significance of tumor-associated neutrophils in colorectal cancer***
Maria R. Galdiero, Italy

32 ***Cellular uptake and degradation of allergens inside dendritic cells.***
Joost Smit, Netherlands

33 ***Clusterin modulates allergic airway inflammation by attenuating CCL20-mediated dendritic cell recruitment***
Hyouk-Soo Kwon, Korea

34 ***Vitamin D3 down regulates the high-affinity receptor for IgE (FcepsilonRI) on human dendritic cells***
Thomas Bieber, Germany

35 ***Evidence that basophil-derived tumor necrosis factor can enhance certain innate or adaptive immune responses in mice.***
Adrian Pilponsky, United States

Lymphocytes and Mediators of Immunoregulation

36 ***Role of costimulatory signals in the induction of T cell steroid resistance in asthma***
Akio Mori, Japan

37 ***IL-4 abrogates T(H)17 cell-mediated inflammation by selective silencing of IL-23 in antigen-presenting cells***
Emmanuella Guenova, Switzerland

38 ***Platelets influence B cells via CXCL12***
Connor F. Alexander, United States

39 ***Cellular crosstalk between airway epithelial and endothelial cells augments CX3CL1 shedding during viral infections***
Donna E. Daives, United Kingdom

40 ***Activating Transcription Factor 3 (ATF3) causes susceptibility to opportunistic infections during post-septic immunosuppression***
Wolfram Hoetzenecker, Switzerland

41 ***Lymphoproliferative disease in the setting of Serine/Threonine Kinase 4 (STK4) defect***
Michelle Tseng, United States

Tuesday, 5 April 2016

7:00 – 8:00 **Life in Science Breakfast Discussion** . . . . . Pinckney Room
Dr. Johannes Ring, Germany

7:00 – 13:00 **Registration Open** . . . . . Registration Desk, Mezzanine Level

7:00 – 13:00 **Speaker Ready Room Open**. . . . . Laurens Room

8 :00 – 9 :45 **Oral Abstract Session 3** . . . . . Carolina Ballroom
IgE: Its Discovery and Roles in Allergy
Chairpersons: Thomas Bieber, Germany
Hans-Uwe Simon, Switzerland

8:00 ***The Discovery of IgE***
Toskiaki Kawakami, Japan

8:30 ***The Discovery of IgE***
S.G.O. Johansson, Sweden

9.00 ***The Implications of Identifying IgE in Allergy***
Thomas Platts-Mills, United States

9:45 – 10:30 **Break**. . . . . .

10:30 – 13:00 **Oral Abstract Session 4** . . . . . Carolina Ballroom
Pathophysiology of Allergic Disorders; Barrier Function, Inflammation and Remodeling
Chairpersons: Mubeccel Akdis, Switzerland
Judah Denburg, Canada

10:30 **42** ***Lipid mediators in asthma: defining targets and biomarkers in severe asthma cohorts and experimental medicine studies***
Sven-Erik Dahlen, Sweden

10:47 **43** ***Hemopoietic progenitor expression of epithelial cytokine receptors in allergic asthma: role of Toll-like receptors***
Damian Tworek, Canada

11:04 **44** ***Role of group 2 innate lymphoid cells and IL-13 in bronchial epithelial cell tight junction barrier leakiness***
Cezmi Ali Akdis, Switzerland

11:21 **45** ***Allergenic proteases cleave the chemokine CX3CL1 directly from the surface of airway epithelium and augment the effect of rhinovirus***
Matt Loxham, United Kingdom

11:38 **46** ***Epithelial barrier defects and eosinophil extracellular trap formation in active eosinophilic esophagitis***
Dagmar Simon, Switzerland

11:55 **47** ***Mechanisms of inducing and breaking allergen tolerance***
Mubeccel Akdis, Switzerland

12:12 **48** ***Characterization of allergen, chloroquine (CQ) and histamine-sensitive "Itch Nerves" terminating in mouse skin***
Bradley J. Undem, United States

12:29 **49** ***Enzymatically active sADAM33 is increased in asthma and causes airway remodeling without inflammation***
Hans Michael Haitchi, United Kingdom

12:46 **50** ***sADAM33 causes airway (P)remodeling in early life and enhances eosinophilic airway inflammation and bronchial hyperresponsiveness***
Elizabeth R. Davies, United Kingdom

13:00 – 14:30 **Lunch** . . . . . Colonial Room

16:30 – 19:00 **Boat Ride** . . . . . .

19:00 – 22:00 **Informal Dinner**. . . . . .

Wednesday, 6. April 2016

7:00 – 8:00 **Life in Science Breakfast Discussion** . . . . . Pinckney Room
Dr. Patrick Holt, Australia

7:00 – 13:00 **Registration Open** . . . . . Registration Desk, Mezzanine Level

7:00 – 13:00 **Speaker Ready Room Open** . . . . . Laurens Room

8:00 – 10:00 **Oral Abstract Session 5** . . . . . Carolina Ballroom

Translation of Pathophysiology of Allergic Disorders to Novel Preventative and Therapeutic Interventions
Chairpersons: Sven-Erik Dahlen, Sweden
Johannes Ring, Germany

8:00 **51** ***The allergic pre-commitment of the airways***
Ulrich M. Zissler, Germany

8:17 **52** ***Predisposition to asthma due to environmental priming of natural killer cells in utero***
Magdalena M. Gorska, United States

8:34 **53** ***Interleukin-36γ: A novel target for asthma?***
Hock L. Tay, Australia

8:51 **54** ***Acute lipopolysaccharide exposure during a severe paramyxoviral respiratory infection is sufficient to prevent development of post-viral atopic disease***
Mitchell H. Grayson, United States

9:08 **55** ***The potential for repositioning anti-thyroid agents as anti-asthma drugs***
Kenji Izuhara, Japan

9:25 **56** ***Glucagon like peptide-1 receptor signaling inhibits both lung innate and adaptive immunity mediated allergic inflammation to inhaled aeroallergen***
R. Stokes Peebles, Jr., United States

9:42 **57** ***Early introduction of egg with two step procedure for infants with atopic dermatitis to prevented egg allergy: A double-blind placebo-controlled randomized clinical trial***
Yukihiro Ohya, Japan

10:00 – 10:30 **Break** . . . . .

10:30 – 12:00 **Oral Abstract Session 6** . . . . . Carolina Ballroom

Allergens and Precision Diagnosis of Allergy
Chairpersons: Barbara Bohle, Austria
Martin D. Chapman, United States

10:30 **58** ***Heterogeneity of airway Type 2 innate lymphoid Cells (ILC2s) and their steroid responsiveness in asthma***
Rafeul Alam, United States

10:47 **59** ***Allergen endotoxins induce T cell-dependent and non-IgE-mediated nasal hypersensitivity in mice***
Tomohiro Yoshimoto, Japan

11:04 **60** ***The interplay of Bet v 1 and birch pollen lipids in the allergic sensitization process***
Barbara Gepp, Austria

11:21 **61** ***Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence – a BAMSE/MeDALL study***
Marianne van Hage, Sweden

11:38 **62** ***Component-based analysis of IgG4 antibodies suggests that the low capacity of the Immuno Solid-Phase Allergen Chip microarray limits detection of specific IgE antibodies in patients with eosinophilic esophagitis***
Elizabeth A. Erwin, United States

Wednesday, 6. April 2016, *continued*

12:00 – 13:00 **Paul Kallos Lecture** . . . . . Carolina Ballroom

"Stressed Out: ER Stress in Immunity and Disease"

Dr. Glimcher is the Stephen and Suzanne Weiss Dean of Weill Cornell Medical College in New York, where she is also Professor of Medicine. In addition, she is Provost for Medical Affairs of Cornell University. Previous to her current positions, she was the Irene Heinz Given Professor of Immunology at the Harvard School of Public Health, where she was director of the Division of Biological Sciences, and Professor of Medicine at Harvard Medical School, where she headed the immunology program. She also served as Senior Physician and Rheumatologist at the Brigham and Women's Hospital. As an immunologist, her primary research interests are elucidating the molecular pathways that regulate CD4 T helper cell development and activation, critical for both the development of protective immunity and for the pathophysiologic immune responses underlying autoimmune, infectious and malignant diseases. She is a Fellow of the American Academy of Arts and Sciences, a Member of the Institute of Medicine of the National Academy of Sciences and a Member of the National Academy of Sciences. She sits on the Board of Trustees of Cornell University, the Board of Overseers of Weill Cornell Medical College, the Board of Trustees of Memorial Sloan Kettering Cancer Center and the Board of Directors of the New York Blood Foundation and is on the Corporate Board of Directors of the Bristol-Myers Squibb Pharmaceutical Corporation and the Waters Corporation.

Dr. Glimcher speaks nationally and internationally on rheumatology, immunology, skeletal biology and translational medicine and has contributed more than 350 scholarly articles and papers to the medical literature.

13:00 – 14:30 **Lunch** . . . . . Colonial Room

17:00 – 19:00 **Registration Open** . . . . . Registration Desk, Mezzanine Level

17:00 – 19:00 **Poster Session** . . . . . Gold Ballroom, Calhoun Room

Conventional and Novel Biomarkers of Allergy, Asthma and COPD, Allergens and Diagnosis of Allergy, Allergen Specific Immunotherapy

Chairpersons: Judith A. Woodfolk, United States
Parameswaren Nair, Canada
Heimo Breiteneder, Austria
Peter S. Creticos, United States
Stephen R. Durham, United Kingdom
Robyn O'Hehir, Australia

Conventional and Novel Biomarkers of Allergy

63 ***Relation of serum and sputum IL-33 levels with sputum inflammatory cells and lung function in bronchial asthma***
Young Joo Cho, Korea

64 ***CD48 and asthma: association and potential biomarker***
Francesca Levi-Schaffer, Israel

65 ***Potential biomarkers representing subtypes of aspirin exacerbated respiratory disease (AERD)***
Hae-Sim Park, Korea

66 ***MicroRNA-146a and microRNA-155 expression in induced sputum and blood of allergic asthmatics***
Madeleine Rådinger, Sweden

67 ***Distinct plasma chemokine levels in non-IgE-mediated gastrointestinal food allergy, compared with IgE-mediated food allergy***
Kanami Orihara, Japan

68 ***Biomarkers in nasopharyngeal aspirates at first wheezing episode to predict recurrent wheezing***
Kazuko Sugai, Japan

Allergens and Diagnosis of Allergy

71 ***Towards the identification of IgE antibody binding epitopes in Group 1 mite allergens***
Anna Pomés, United States

72 ***Isoallergen distribution in affinity-purified natural dust mite allergens***
Peter Briza, Austria

Wednesday, 6. April 2016, *continued*

73 ***Are dust mite allergens more abundant or more stable than other Dermatophagoides pteronyssinus proteins?***
Geoffrey A. Mueller, United States

74 ***Lipophilic allergens: An underestimated risk in lupine?***
Uta Jappe, Germany

75 ***Structural, functional and immunologic characterization of profilins from ragweed and mugwort***
Maksymilian Chruszcz, United States

76 ***The novel cat lipocalin Fel d 7 and its cross-reactivity with the dog lipocalin Can f 1: Structural characterization and allergenic activity***
Carl Hamsten, Sweden

77 ***Metabolomic analysis of aqueous pollen extract by nuclear magnetic resonance (NMR)***
Peter M. Thompson, United States

78 ***Clonal analysis of antibody repertoires during treatment with fast-dissolving grass sublingual allergy tablet***
Peter S. Andersen, Denmark

79 ***Macrophage secrete discrete amount of cortisol, which can be captured by the lipocalin-allergen Bos d 5, thereby influencing Lipocalin-2 (LCN2) expression***
Franziska Roth-Walter, Austria

80 ***Conformational IgE-binding epitopes of Ara h 2 and Ara h 6***
Stephen C. Dreskin, United States

81 ***Molecular analysis of IgE antibody responses to shrimp allergens in patients from different geographic regions***
Jonas Lidholm, Sweden

82 ***Histamine release from passively sensitized human basophils and the impact of allergen specific IgE***
Bettina M. Jensen, Denmark

Allergen Specific Immunotherapy

83 ***A randomized controlled trial of intradermal grass pollen immunotherapy for seasonal allergic rhinitis***
Stephen R. Durham, United Kingdom

85 ***Comparison of relative and absolute treatment differences between Sublingual Immunotherapy Tablets and Pharmacotherapies for seasonal and perennial allergic rhinitis: pooled analyses of clinical trials***
Peter S. Creticos, United States

86 ***Timothy grass pollen SLIT tablets' SPT potency compared to a US reference extract***
Désirée E. Larenas Linnemann, Mexico

87 ***Allergen-specific B cell responses to allergen-tolerance induction are characterized by expansion of BR1 cells, IgG4-class switch recombination and CCR5 expression.***
Willem Van de Veen, Switzerland

88 ***Macrophages specifically release cortisol and IL-10 under IgG4 Stimulation; Possible explanation of allergen immunotherapy***
Erika Jensen-Jarolim, Austria

89 ***T cell immunogenicity of major and minor grass pollen allergens***
Peter A. Würtzen, Denmark

90 ***Humanized mice as in vivo model for allergen-specific immunotherapy of IgE-mediated allergy***
Barbara Bohle, Austria

Thursday, 7, April 2016

7:00 – 8:00 **Life in Science Breakfast Discussion** . . . . . Pinckney Room
Dr. Robyn O'Hehir, Australia

7:00 – 13:30 **Registration Open** . . . . . Registration Desk, Mezzanine Level

7:00 – 13:30 **Speaker Ready Room Open** . . . . . Laurens Room

8:00 – 10:15 **Oral Abstract Session 7** . . . . . Carolina Ballroom
Mast Cells, Basophils and Eosinophils in Health and Disease
Chairpersons: Jean S. Marshall, Canada
Marcus Maurer, Germany

8:00 **91** ***Mast cell activation induced by T cell-derived microvesicles: a possible role for miR 4443***
Yospeh A. Mekori, Israel

8:17 **92** ***The mast cell-clock regulates blood histamine levels: implication for stress-induced exacerbation of allergy***
Atsuhito Nakao, Japan

8:34 **93** ***A chimeric protein containing the C-terminus of Bet v 1 is a potent inducer of basophil degranulation***
Heimo Breiteneder, Austria

8:51 **94** ***Activation of human basophils by epithelial cells: a role for IgE Interaction with Galectin-3***
John T. Schroeder, United States

9:08 **95** ***Role of kinases, dynamin and the cytoskeleton in regulating Siglec-8 engagement-induced endocytosis, reactive oxygen species (ROS) production and apoptosis in primary human eosinophils***
Bruce S. Bochner, United States

9:25 **96** ***Eosinophil cytolysis occurs through necroptosis***
Hans-Uwe Simon, Switzerland

9:42 **97** ***IgE antibodies, FceRIa and IgE-mediated local anaphylaxis can limit snake venom toxicity***
Stephen J. Galli, United States

9:59 **98** ***Mast cells actively participate in tumor-promoting inflammation***
Karin Hartmann, Germany

10:15 – 11:00 **Break** . . . . .

11:00 – 12:00 **Oral Abstract Session 8** . . . . . Carolina Ballroom
Biomarkers of Asthma and Allergic Airways Disease
Chairpersons: Kenji Izuhara, Japan
Ruby Pawankar, Japan

11:00 **99** ***Sputum cytokine signature in patients with severe asthma and local autoimmunity***
Manali Mukherjee, Canada

11:17 **100** ***The role of miR-328 in allergic airway disease***
Hock L. Tay, Australia

11:34 **101** ***Global microRNA binding fine-tunes the transcriptome but profoundly alters the translatome: the application of RibomiR-seq to the understanding of epithelial cell activation in severe asthma***
Rocio T. Martinez-Nunez, United Kingdom

11:51 **102** ***Comprehensive evaluation of serum periostin as a phenotype-specific biomarker in asthma***
Anna J. James, Sweden

Thursday, 7. April 2016, *continued*

12:00 – 13:00 **Relaxing Lecture** . . . . . Carolina Ballroom

"Magnolia Gardens; and the effort to Preserve Older Azalea and Camellia Varieties"
Tom Johnson is a lifelong horticulturist whose penchant for sharing horticulture with his professional colleagues and friends has taken him to Europe and Caribbean.

Tom's love of working with plants developed in his youth on a middle-Georgia truck farm. As a member of the Future Farmers of America in high school, Tom oversaw the redesign of downtown Perry, Georgia. The landscaping project won for the city a prestigious national award.

At age 16, Tom went to work for a local garden center and landscaping company. After high school, he attended Abraham Baldwin Agricultural College where he majored in plant propagation.

In 1985, when President Jimmy Carter began building his presidential library in Atlanta, he enlisted Tom to help oversee the building of the gardens. That experience lead to Tom being selected for the design team for the construction of the Evan Allen III Pavilion and the Cecil B. Day Chapel.

During the development of the Carter Center Gardens, Tom apprenticed for five years under world-renowned Japanese architect Kinsako Nakane. Tom is among a handful of Americans to have had this opportunity. Later, Tom worked with Shiro Nakane, Kinsako's son.

After a decade at the Carter Presidential Center, Tom returned to middle Georgia to as the national horticulturist with the American Camellia Society. For the next eight years, he managed the society's camellia collection at Massee Lane Gardens, the society's national headquarters. In that role, Tom traveled the "Southern camellia belt" advising garden and camellia growers on cultural and propagation issues. He also gave camellia lectures and passionately advocated for a need to preserve older varieties.

While at Massee Lane, Tom was approached by John Drayton Hastie Jr., one of the owners of Magnolia Plantation and Gardens in Charleston. Hastie had attended one of Tom's lectures. Tom's presentation was not limited to the preservation of older azaleas and camellias. He also shared his affection for romantic-style gardens.

Hastie was so impressed that he pursued Tom for the next several years, finally convincing him to become Magnolia's executive director. At Magnolia, Tom's responsibilities include the restoration of America's oldest romantic-style garden.

Tom is charged with returning Magnolia to the vision the Rev. John Grimké Drayton had when he designed the gardens for his homesick bride in the mid-1800s. This project launched Tom on a worldwide search for azalea and camellia varieties that predate the 1900s. It also spurred him to share his gardening expertise with colleagues in Belgium, France, Barbados and Cuba.

Tom is a sought-after speaker across the South. He uses his Southern charm and humor to promote the preservation of azalea and camellia collections around the world.

When Tom is asked about his mission at Magnolia, he states simply: "Magnolia is a grand old lady. My job is to shine her shoes, dress her in some new robes, and get her ready for the thousands of suitors that come calling each year. I can think of no better place to finish my career."

13:00 – 13:30 **CIA Business Meeting** . . . . . Carolina Ballroom

13:30 – 15:00 **Lunch** . . . . . Colonial Room

15:00 – 17:30 **Registration Open** . . . . . Registration Desk, Mezzanine Level

15:00 – 17:30 **Poster Session** . . . . . Gold Ballroom, Calhoun Room

Treatment of Immune Disorders, Pathophysiology of Allergic Disorders and Inflammation, Urticaria and Angioedema, Clinical Aspects of Allergic Disorders

Chairpersons: Motohiro Ebisawa, Japan
Bruce S. Bochner, United States
Eva Untersmayr-Elsenhuber, Austria
Allen P. Kaplan, United States
Hae-Sim Park, Korea
Thomas Platts-Mills, United States

Thursday, 7, April 2016, *continued*

Treatment of Immune Disorders

103 ***Usefulness of very low dose oral food challenge and oral immunotherapy***
Motohiro Ebisawa, Japan

104 ***Dual Variable Domain Antibodies, a novel platform for delivery of transformational efficacy to patients***
Melanie Ruzek, United States

105 ***Blood eosinophils predict therapeutic effects of a GATA3 specific DNAzyme on both allergic early and late phase reactions in patients with asthma***
Norbert Krug, Germany

106 ***CompEx: A novel surrogate asthma exacerbation endpoint***
Malin Fagerås, Sweden

107 ***Probiotics attenuate mite allergen-induced allergic inflammation via PPARγ in the murine model of allergic diseases***
Jiu-Yao Wang, Taiwan

108 ***Direct modulation of dendritic and epithelial cell responses by human milk oligosaccharides.***
Paul Forsythe, Canada

110 ***Protection from food allergy development is associated with distinct characteristics in an oral mouse immunization protocol***
Eva Untersmayr-Elsenhuber, Austria

Pathophysiology of Allergic Disorders and Inflammation

111 ***Skin barrier homeostasis and its failure in atopic disorders***
Masayuki Amagai, Japan

112 ***Inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5) affects epidermal morphology in constitutive knockout mice and could be a novel key player in delayed type hypersensitivity responses of the skin***
Sebastian Huth, Germany

113 ***Epicutaneous allergic sensitization by synergy between allergen protease-activity and mechanical skin barrier damage in mice***
Toshiro Takai, Japan

114 ***Human rhinovirus infected epithelial cells produce chemoattractants for fibroblasts***
David Proud, Canada

115 ***Vertical transmission of respiratory syncytial virus (RSV) to fetuses in utero alters post-natal Th1 and Th2 cytokine levels in weanling rats re-challenged with RSV***
Giovanni Piedimonte, United States

116 ***Interleukin-13 induces glucocorticoid-insensitive hyperreactivity of human small airways***
Mikael Adner, Sweden

117 ***Pollen extracts and cat dander extract require Myeloid Differentiation Factor 2 (MD2) and MyD88 adaptor to stimulate innate neutrophil-mediated allergic sensitization***
Sanjiv Sur, United States

118 ***Water soluble chitosan inhibits nerve growth factor in murine model of mite-allergen induced allergic rhinitis***
Jiu-Yao Wang, Taiwan

119 ***Insulin regulates proteinase-activated receptor-2 (PAR2) expression on airway epithelium***
Vivek Gandhi, Canada

120 ***Roles of C-type lectin receptors Dectin-1/2 in house dust mite-induced allergic airway inflammation***
Hiroshi Nakajima, Japan

121 ***Allergen-exposed bronchial epithelial cell-stimulated migration of smooth muscle is suppressed by inhaled corticosteroid and long-acting beta agonist therapy***
Margaret M. Kelly, Canada

122 ***Immunoglobulin G to 91 allergenic molecules in early childhood by route of exposure, current and future allergic sensitization: a MAS birth cohort study***
Paolo M. Matricardi, Germany

123 ***Activation of peroxidases causes airway inflammation via production of hypothiocyanite***
Masahiro Ogawa, Japan

Thursday, 7. April 2016, *continued*

124 ***Targeting Nrf2 by triterpenoid CDDO-Me inhibits IL-33 secretion and improves Th2-type airway inflammation induced by natural airborne allergen exposure***
Masaru Uchida, Japan

125 ***Leukotriene C4 Induces Pulmonary Eosinophilia by an Interleukin 33-Driven Mechanism***
Joshua Boyce, United States

126 ***Involvement of cysteinyl leukotriene 2 receptors in airway allergic inflammation in mice***
Hiroyuki Tanaka, Japan

127 ***The regulatory role of a novel activating receptor in mast cell- and IgE-dependent anaphylaxis***
Jiro Kitaura, Japan

128 ***Basolateral sphingosine-1-phosphate stimulation influences barrier integrity of intestinal epithelial cells***
Eva Untersmayr-Elsenhuber, Austria

129 ***Increased Expression of Filaggrin in Nasal Polyps as compared to Nasal mucosa of patients with allergic rhinitis***
Ruby Pawankar, Japan

130 ***Analysis of transcriptional regulation for periostin***
Yasutaka Mitamura, Japan

Urticaria, Anaphylaxis and Angioedema

131 ***Educational program anaphylaxis school: contents, methods, target groups***
Johannes Ring, Germany

132 ***Increased plasma concentrations of angiogenic and lymphangiogenic factors in patients with hereditary angioedema with C1-INH deficiency***
Stefania Loffredo, Italy

133 ***Condition of hypoxia, cytokine or estrogen stimulation of endothelial cells augments activation of the surface-bound prekallikrein-high molecular weight kininogen complex and releases urokinase: Implications for hereditary angioedema (HAE).***
Allen P. Kaplan, United States

134 ***Idiopathic angioedema: Difficult cases and uncommon findings***
Oral Alpan, United States

135 ***Sustained effect and clinical outcomes in chronic spontaneous urticaria in patients receiving Omalizumab for several years***
Marta Ferrer Puga, Spain

136 ***Effects of an oral CRTH2 antagonist on CRTH2-bearing blood leukocytes in chronic spontaneous urticaria patients***
Sarbijt S. Saini, United States

Clinical Aspects of Allergic Disorders

137 ***Sensitization to mouse (Mus m 1) is a leading pattern in Amaxhosa atopic dermatitis patients in Cape Town Region, South Africa.***
Peter Schmid-Grendelmeier, Switzerland

139 ***Prevalence of mediator-related symptoms in patients with mastocytosis and response to anti-mediator treatments***
Massimo Triggiani, Italy

140 ***Barriers to the use of human tissue in asthma research***
Joanna M. Edwards, United Kingdom

141 ***Innovative nasal filters allow for allergen exposure monitoring and are acceptable to ware***
James P. Hindley, Untied Kingdom

142 ***Identifying immune deficiencies by curbside consultation; iCurb***
Oral Alpan, United States

143 ***Bleach Baths Imrpove Atopic Dermatitis, Reduce Itch and Repair Barrier***
Lisa A. Beck, United States

18:15 – 22:15 **Gala Dinner at Middleton Place**

Friday, 8, April 2016

7:00 – 8:00 **Life in Science Breakfast Discussion** . . . . . Pinckney Room
Dr. Kathleen Barnes, United States

7:30 – 13:00 **Registration Open** . . . . . Registration Desk, Mezzanine Level

7:30 – 13:00 **Speaker Ready Room Open** . . . . . Laurens Room

8:00 – 9:00 **CIA Council Meeting** . . . . . Pinckney Room

9:00 – 10:30 **Oral Abstract Session 9** . . . . . Carolina Ballroom

Targeted Treatment of Allergic and Immune Disorders
Chairpersons: Paul Foster, Australia
Eugene Bleecker, United States

9:00 **144** ***Omalizumab reduces bronchial mucosal inflammation and improves lung function in non-atopic asthma***
Christopher J. Corrigan, United Kingdom

9:17 **145** ***Omalizumab is effective and safe in symptomatic dermographism and cold urticarial***
Martin Metz, Germany

9:34 **146** ***Inhaled budesonide induces expression of genes involved in transcription***
Richard Leigh, Canada

9:51 **147** ***Preventive and therapeutic amelioration of food allergy by inhibitors of histamine-releasing factor***
Toshiaki Kawakami, United States

10:08 **148** ***Gauging Response in Allergic rhinitis to Sublingual and Subcutaneous immunotherapy (GRASS): nasal allergen challenge and local and systemic immunologic responses***
Stephen R. Durham, United Kingdom

10:25 **149** ***Circulating CD4$^+$CXCR5$^+$PD-1$^+$FoxP3$^+$ T follicular regulatory cells are decreased in allergic rhinitis and restored following subcutaneous and sublingual Immunotherapy***
Mohamed H. Shamji, United Kingdom

10:30 – 11:00 **Break** . . . . .

11:00 – 13:00 **Oral Abstract Session 10** . . . . . Carolina Ballroom

The Interplay Between T Lymphocytes, B Lymphocytes and Macrophages in Allergy and Its Treatment
Chairpersons: Susan MacDonald, United States
R. Stokes Peebles, United States

11:00 **150** ***Pathogenic Th2 (Tpath2) cells in airway inflammation***
Toshinori Nakayama, Japan

11:17 **151** ***Negative feedback mechanisms for ILC2 functions and type 2 innate immune responses***
Shigeo Koyasu, Japan

11:34 **152** ***Follicular Helper T (Tfh) Cells Mediate IgE Antibody Responses to Airborne Allergens***
Hirohito Kita, United States

11:51 **153** ***CCL17 induces accumulation of myeloid dendritic cells (DC) and hyperresponsiveness in allergic airway inflammation***
Moyar Q. Ge, United States

12:08 **154** ***New insights into T-Cell Immunity to rhinovirus in atopic asthma***
Judith A. Woodfolk, United States

12:25 **155** ***Ovarian hormones increase ILC2 cytokine expression and Alternaria extract-induced airway inflammation***
Dawn C. Newcomb, United States

12:42 **156** ***Increase in activated Group 2 innate lymphoid cells in the airways of mild asthmatics following allergen inhalation challenge***
Roma Sehmi, Canada

-1-

Progenitors highly committed to the mast cell lineage identified in human blood

Joakim S. Dahlin[1,2], Andrei Malinovschi[3], Helena Öhrvik[1], Martin Sandelin[3], Christer Janson[3], Kjell Alving[4] and Jenny Hallgren[1]
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[2]Present address: Clinical Immunology and Allergy Unit, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden.
[3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[4]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

In mice, committed mast cell progenitors originate from the bone marrow, circulate in the blood and mature into distinctive subtypes in the peripheral tissues. Human mast cells are also divided into subtypes based on location and expression of certain mast cell proteases. However, whether committed human mast cell progenitors exist has remained unknown. In our study we used fluorescence activated cell sorting (FACS) of human blood cell populations that were subsequently cultured to identify novel cell populations with high mast cell forming capacity. Studies by Kirshenbaum et al have revealed mast cell/monocyte potency in $CD34^+$ $CD117^+$ $CD13^+$ blood cells. To facilitate our study of rare cell populations, monocytes, T cells and B cells were excluded from the analysis by the use of lineage (Lin) markers. Interestingly, Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $CD13^+$ blood cells could be grouped into cell populations that either were expressing FcεRI or not, with the highest mast cell capacity demonstrated in the $FcεRI^+$ fraction. When the Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $FcεRI^+$ cells were gated and analyzed for CD13 expression, these cells had variable expression of CD13. Therefore, the Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $FcεRI^+$ cells (irrespective of CD13 expression) were cultured in a myeloerythroid cytokine cocktail for 7 days. We found that the progeny had a mast cell-like appearance with granules and a high fraction retained CD117 and FcεRI expression. The primary Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $FcεRI^+$ cells expressed integrin β7. Whole-transcriptome microarray analysis of their relative expression in comparison to blood basophils revealed that they expressed higher levels of *Hpgds*, *Kit* and *Hdc*. The genes coding for mast cell tryptases and carboxypeptidase A also appeared to be expressed at higher levels in these cells, and mRNAs for *Tpsab1*, *Tpsb2* and *Cpa3* could be detected by quantitative reverse transcriptase PCR. Single cell analyses of the Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $FcεRI^+$ cells revealed that they were dividing slowly; each cell became in median 3 cells in 7 days. Altogether, we conclude that the Lin^- $CD34^{hi}$ $CD117^{int/hi}$ $FcεRI^+$ blood cells are highly committed to the mast cell lineage and propose that they constitute a committed mast cell progenitor pool in human blood.

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Rab12 regulates retrograde transport of mast cell secretory granules by interacting with the RILP-dynein complex

Adi Efergan[1], Nurit P. Azouz[1]*, Ofir Klein[1], Efrat Rosenbaum[1], Kenta Noguchi[2], Marc E. Rothenberg[3], Mitsunori Fukuda[2] and Ronit Sagi-Eisenberg[1]
[1]Department of Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel, [2]Laboratory of Membrane Trafficking Mechanisms, Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aobayama, Aoba-ku, Sendai, Miyagi 980-8578, Japan and [3]Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio, USA.

Background: Secretory granule (SG) transport is a critical step in regulated exocytosis including degranulation of activated mast cells. The latter process results in the secretion of inflammatory mediators that are prestored in the mast cell SGs, thereby enhancing immune responses such as in allergy and host defenses. In mast cells, the SGs move bidirectionally on the microtubule (MT) network. However, precisely how SG transport is coupled to the MTs remains obscure. Rab GTPases are master regulators of vesicular trafficking that mediate vesicle transport. Therefore, unveiling the Rab networks associated with mast cell degranulation provides invaluable tools for decoding the mechanisms involved in this process. Along this line of thoughts, we recently developed a high-resolution imaging-based methodology that allowed screening Rab GTPases for their phenotypic and functional features. This screen identified 30 Rabs as general regulators of exocytosis in the widely studied mast cell line, RBL-2H3 [Azouz et al., J. Immunol. **189,** 2169-2180, (2012)]. Intriguingly, our screen identified Rab12, one of the less characterized Rab GTPases, as a potential regulator of SG transport.

Method: To explore the role of Rab12 in regulating mast cell exocytosis and decipher Rab12 underlying mechanism of action, we combined high-resolution live cell confocal imaging with biochemical methodologies.

Results: We show that knockdown of Rab12 potentiates mast cell exocytosis, whereas conversely, expression of a constitutively active Rab12 mutant (CA Rab12) inhibits this process. We also show that Rab12 is activated in a receptor dependent fashion and both the FceRI- activated Rab12 or the CA Rab12 mutant promote perinuclear clustering of the SGs. Finally, we show that the RILP-dynein complex mediates this perinuclear accumulation of the SGs and have identified RILP as a novel, heretofore unrecognized effector of Rab12.

Conclusions: Collectively our results assign Rab12 a key role in regulating mast cell secretion by controlling the retrograde transport of the SGs.

-3-

Interleukin-33 induces phenotypic changes in human skin-derived mast cells

Magda Babina, PhD, Sven Guhl, MS, Torsten Zuberbier, MD

Background: Mast cells (MCs), the effector cells of IgE-mediated allergic disorders and other pathophysiological processes, are of hematopoietic origin but undergo terminal differentiation in contact with their target tissues like the skin. The composition of the immediate micromilieu will therefore shape MC phenotypes. SCF is arguably the most decisive growth and differentiation factor of the lineage, but more recently, IL-33 has likewise leaped into prominence as a MC-supportive mediator. So far, studies have mainly employed MC lines, immature MCs or MCs generated from hematopoietic precursor cells. Enhanced IL-33 levels are associated with chronic skin diseases in humans, so information on skin-derived MCs seems of particular pathophysiological value, especially in the setting of long-term exposure to IL-33.

Methods: We used our well-established technique to purify MCs to homogeneity from human skin tissue. MCs were exposed to SCF alone or SCF combined with IL-33 (SCF/IL-33) for up to 5 weeks. Since IL-4 is a supportive factor of human skin-derived MCs in the presence of SCF, the results for SCF/IL-33 were contrasted against those for SCF/IL-4. We studied cell expansion, content of preformed mediators, expression of MC related genes, FcεRI protein and histamine release (HR) triggered by FcεRI aggregation or Substance P (SP).

Results: IL-33 supported MC expansion (in part by counteracting apoptosis), and almost reproduced the behavior of IL-4. IL-33, like IL-4, substantially increased histamine content per cell, and this was accompanied by enhanced expression of the transcript for histidine decarboxylase. In stark contrast to IL-4, however, IL-33 dampened FcεRI expression and the FcεRIα transcript. Intriguingly, HR triggered by FcεRI aggregation was negatively or positively affected depending on donor, though a decline was more common (9 down/4 up), while SP-mediated HR was consistently and profoundly decreased by IL-33.

Conclusions: IL-33 has ambivalent effects on skin-derived MCs. While enhancing MC expansion and histamine content, IL-33 negatively regulates FcεRI expression and more commonly lessens degranulation responses. The latter may serve as a negative feedback to chronic exposure to "alarmins" like IL-33. Collectively, cutaneous IL-33 may shape MC phenotypes and contribute to MC variability across individuals and disease entities alike.

-4-

Lipid mediator metabolic profiling after IgE-induced activation of two distinct mast cell populations

Alexander Fauland[1,3], Maria Ekoff[2] , Craig Wheelock[1,3], Gunnar Nilsson[2,3]
Departments of [1]Medical Biochemistry and Biophysics and [2]Medicine; and [3]The Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden.

Background: Upon activation, e.g., IgE-receptor aggregation, mast cells have the capacity to release a wide variety of mediators. Eicosanoids are made by oxidation of twenty-carbon essential fatty acids and are divided into four main families: the prostaglandins, prostacyclins, thromboxanes and leukotrienes. The cys-leukotrienes C_4, D_4 and E_4, as well as the prostaglandin D_2 are well known mast cell mediators, mediating symptoms in e.g., allergy and asthma.

Objective: The objective of this study was to investigate, using a mass spectrometric-based approach, the secretion of lipid mediators representing the cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways in two different mast cell populations after treatment either with the calcium ionophore A23187, or with the more physiological relevant IgE+antigen or IL-33.

Methods: Mouse bone marrow cultured cells were differentiated into either mucosal like mast cells (MLMC) or connective tissue like mast cells (CTLMC). They were treated with either calcium ionophore A23187, IgE+antigen or IL-33. Supernatants were taken 2, 8, 15 and 60 min after treatment and analyzed with LC-MS/MS.

Results: After correction for reproducibility and false positivity due to media contaminations 20 mediators were found to be above the limit of quantification. The lipid mediators originated predominantly from arachidonic acid, but some also from dihomo-gamma-linolenic acid and eicosapentaenoic acid. Activation with IgE+antigen caused a rapid secretion of 5-LOX products, including LTB_4 and LTC_4, which in MLMC was higher than that induced by the calcium ionophore A23187. In CTLMC the reverse was observed, with more 5-LOX products after A23187 treatment than after IgE+ag. The predominant COX product secreted from both MLMC and CTLMC was PGD_2, but also substantial amounts of TxB_2 and 12-HHTrE were secreted. IL-33 did not induce secretion of any of the mediators analyzed, neither in MLMC nor in CTLMC.

Conclusion: This study reveals the capacity of mast cells to secrete a high variety of lipid mediators and also a difference in the ability between mast cell phenotypes to generate these lipids, which may have biological implications in mast cell-mediated regulation of inflammatory responses.

-5-

IL-6 promotes an increase in human mast cell number and reactivity through suppression of SOCS3

Avanti Desai[1], Mi-Yeon Jung[1], Ana Olivera[1], Alasdair M. Gilfillan[1], Calman Prussin[1], Arnold S. Kirshenbaum[1], Michael A. Beaven[2], and Dean D. Metcalfe[1]. [1]Laboratory of Allergic Diseases, NIAID; [2]Laboratory of Molecular Immunology NHLBI, NIH, Bethesda, MD 20892, USA

Rationale: IL-6, which is elevated in association with mastocytosis, asthma and urticaria, is used in conjunction with stem cell factor (SCF) to generate human MCs (HuMCs) from progenitor (CD34+) cells. Despite these associations, the effects on, and mechanisms by which prolonged exposure to IL-6 alters HuMC number and function are not well understood. We thus examined the effect of IL-6 on HuMC function, the mechanisms by which IL-6 exerts its effects, and the relationship of these findings to mastocytosis.

Methods: HuMCs were cultured in SCF with or without IL-6. The results of FceRI aggregation, and the expression of both proteases and receptors, including the soluble IL-6 receptor (sIL-6R), were quantitated. Methylation specific PCR was employed to examine epigenetic changes in SOCS3. Healthy controls and patients with mastocytosis provided serum samples which were assayed for tryptase, IL-6, and sIL-6R.

Results: IL-6 enhanced MC proliferation, maturation, and reactivity following FceRI aggregation. IL-6 reduced expression of SOCS3, which correlated with methylation of the SOCS3 promoter and increased expression and activation of STAT3. IL-6 also suppressed production of sIL-6R. Serum levels of sIL-6R were similarly reduced in patients with mastocytosis.

Conclusions: IL-6 increases mast cell proliferation and formation of a more reactive phenotype enabled by suppressing proteolytic cleavage of sIL-6R from IL-6R and down regulation of the SOCS3 auto-inhibitory pathway. We suggest IL-6 blockade might ameliorate MC related symptoms and pathology in patients with mast cell driven diseases and elevated levels of IL-6.

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Regulation of mast cell function by the association of mitochondrial pyruvate dehydrogenase and microphthalmia transcription factor.

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Recently, we have shown that mitochondrial ATP production is essential for mast cell degranulation and function [1]. Inhibition of mitochondrial ATP production led to a drastic decrease in mast cell degranulation while inhibition of cytosolic ATP production did not cause this effect. We then performed yeast two-hybrid screening using microphthalmia transcription factor (MITF) as bait, and observed that MITF and pyruvate dehydrogenase (PDH), the major regulator of mitochondrial ATP production, are associated. PDH occupies a key position in the oxidation of glucose by linking the glycolytic pathway to the oxidative pathway of the tricarboxylic acid cycle. In mammals, PDH plays the role of a gatekeeper in the metabolism of pyruvate to maintain glucose homeostasis during the fed and fasting states. This led us to hypothesize that PDH-MITF interaction could contribute to the regulation of the mitochondrial ATP production in mast cell degranulation. MITF is best known for its role as a transcription factor, but in the present work we have clearly confirmed our yeast two-hybrid data that MITF is located in mast cell mitochondria where it interacts with PDH.

To see whether MITF has a direct effect on PDH complex activity, we transfected RBL cells with plasmids encoding the full length MITF or mutated MITF (which lacks the leucine zipper domain and thus cannot bind DNA). It was clearly observed that overexpression of either full length or mutated MITF led to a reduction in PDH complex activity, mitochondrial ATP production and oxygen consumption levels.

Moreover, immunoactivation of cultured mast cells caused a decrease in the phosphorylation of PDH complex, elevation in its activity while causing a dissociation of MITF from the complex. The use of CPI-613, a known inhibitor of PDH activity, resulted in increased phosphorylation of the complex and MITF-PDH interaction. Treating mice with CPI-613 for 3 hours reduced their blood histamine release levels by approximately 20%, which indicates the importance of PDH for mast cell function.

Thus, we described here a non-canonical function of MITF unrelated to its DNA binding activity, which is a novel regulator of PDH activity. PDH activity is essential for mast cell function and inhibition of its activity could serve as a new target for the manipulation of allergic diseases.

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Erlich TH, Yagil Z, Kay G, Peretz A, Migalovich-Sheikhet H, Tshori S, Nechushtan H, Levi-Schaffer F, Saada A, Razin E. (2014) Mitochondrial STAT3 plays a major role in IgE-antigen-mediated mast cell exocytosis. J Allergy Clin Immunol. 134: :460

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Mechanism of High-potency Inactivation and Active-site Titration of Mast Cell β-Tryptase by Nafamostat

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Background. β-Tryptases, which are the most abundant proteins stored and secreted by human mast cells, may contribute to defense against pathogens but also are implicated in the pathogenesis of disorders like asthma, arthritis, and inflammatory bowel disease. Upon release from degranulating cells, β-tryptases resist inactivation by serpins and other proteinaceous anti-proteases of blood and interstitial fluids, but can be inactivated by small, drug-like molecules. Among the most potent of these inhibitors is nafamostat, which has been used systemically in humans to treat conditions (like pancreatitis) related to proteases other than tryptases, but also shows efficacy in mouse models of asthma, colitis, itching, in which tryptases are implicated directly. The present study reports results of efforts to define the specificity, potency, duration and mechanism of action of nafamostat as a β-tryptase inhibitor of pharmaceutical interest in allergic airway disease.

Method. To establish relative potencies, we compared inactivation of human β-tryptase, prostasin, matriptase, and airway trypsin-like protease (HAT) by nafamostat, camostat, bis(5-amidino-2-benzimidazolyl)methane (BABIM), aprotinin, and benzamidine.

Results. Nafamostat achieved complete, stoichiometric and very slowly reversible inhibition of matriptase and β-tryptase, but inhibited prostasin less potently and was weakest versus HAT. The IC_{50} of nafamostat's leaving group, 6-amidino-2-naphthol, was $>10^4$-fold higher than that of nafamostat itself, consistent with suicide rather than product inhibition as mechanisms of prolonged inactivation. Stoichiometric release of 6-amidino-2-naphthol allowed highly sensitive fluorometric estimation of active-site concentration in preparations of matriptase and β-tryptase. Camostat, which is a suicide inhibitor mechanistically related to nafamostat, inactivated all enzymes but was less potent overall and weakest towards matriptase, which, however was strongly inhibited by BABIM. Aprotinin was completely ineffective versus β-tryptase but exhibited nearly stoichiometric inhibition of prostasin and matriptase, and was much weaker towards HAT. Benzamidine was universally weak. Thus, each inhibitor profile was distinct.

Conclusions. Nafamostat is a highly potent suicide inhibitor of human β-tryptase effecting durable loss of activity. Because it is attacked stoichiometrically by the proteolytic subunits of the β-tryptase tetramer, while releasing a fluorophore, nafamostat can be used to active-site titrate preparations of β-tryptase. However, nafamostat is only partially selective for tryptase relative to other airway trypsin-like proteases. These data support the therapeutic potential of nafamostat as a β-tryptase inhibitor. Although there is the potential for bystander effects involving other proteases, these effects may not be major drawbacks, given nafamostat's history of safe systemic use in humans.

Support: NIH Grant P01 HL024136.

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IgE-independent human mast cell activation by wheat amylase trypsin inhibitors

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Background: The growing prevalence of Non-celiac gluten sensitivity (NCGS) is accompanied by an increased consumption of wheat α-amylase/trypsin inhibitors (ATI), which is considered to be a trigger of NCGS besides gluten. ATI are proteolysis-resistant molecules that co-purify with the ω-fraction of gliadins in wheat-related species. They appear as monomers, dimers and tetramers. The monomers contain a variable primary structure of 120 to 150 amino acids and a conserved secondary structure that is highly disulfid-linked. After oral ingestion of wheat, ATI can come in contact with immune cells. ATI have proinflammatory effects on macrophages and dendritic cells, but mast cells have not been examined in this respect.

Methods: Isolated human intestinal mast cells (MC) obtained from surgical tissue specimen were challenged with several molecules of the ATI family, each each at 125 µg/mL. Degranulation, leukotrienes synthesis, cytokine expression and phosphorylation of signaling molecules were examined.

Results: The ATI dimer induced histamine release in in MC in a dose and time-dependent manner almost as efficiently as IgE receptor crosslinking (30-40% release). The dimer also induced leukotriene C4 release likely in an IgE receptor-independent manner, since IgE receptor crosslinking enhanced pERK phosphorylation, whereas the dimer reduced it. The ATI tetramer induced the expression of IL-8 and Il-1b mRNA, but not MCP-1 mRNA. The tretramer effects, other than the dimer effects, are likely TLR-4-dependent, since they resembled the LPS effects on MC, and they were only significant in the presence of sCD14, the obligatory TLR4-coreceptor.

Conclusion: Wheat ATI have proinflammatory effects on human MC that are IgE-independent and in part mediated by TLR4. Therefore MC could be involved in the pathogenesis of NCGS.

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The value of whole genome sequencing in identifying genetic determinants of asthma in populations of African ancestry.

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Background: African-admixed populations in the Americas represent one of the most genetically diverse ancestries, but the fine-scale population structure resulting from the African Diaspora has not been well quantified.

Method: We performed whole genome sequencing (WGS) on 642 unrelated individuals of sub-Saharan African ancestry from each of 15 North, Central, and South American plus Caribbean populations and Yoruba-speaking individuals from Ibadan, Nigeria constituting the *Consortium on Asthma among African-ancestry Populations in the Americas* (CAAPA). To discover genes influencing risk for asthma among individuals of African ancestry, we leveraged these WGS data to (1) quantify the complete spectrum of variation observed (~44M SNPs in total) within CAAPA of which 48% of variants have not been previously documented; (2) develop an African Diaspora Power Chip (ADPC), comprising ~750,000 SNPs that recovers all the variation with a minor allele frequency >1% observed in CAAPA much of which is currently unattainable when relying on state-of-the-art genomewide association approaches such as imputation; and (3) genotype >13,000 DNA samples from African-admixed asthmatics and non-asthmatics using the ADPCto identify novel genetic determinants conferring risk of asthma in the single largest study of African ancestry samples to date.

Results: We are currently performing an un-biased GWAS illustrating the advantages of leveraging WGS data in designing the ADPC, as well as the value of these WGS samples as an imputation reference panel.

Conclusions: We anticipate detecting novel regions containing genetic determinants of asthma specific to populations of African ancestry due to the strong limitations with respect to both sample size and inadequacy in tagging of common variation in prior GWAS studies.

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Association of Season of Birth with DNA Methylation and Allergic Disease

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Background: Season of birth has been associated with allergy risk, however the biological mechanisms underlying this observation are unclear. Environmental exposures can induce changes in DNA methylation, with potentially long-lasting effects on gene expression and disease risk. This study examined whether DNA methylation could underlie the association between season of birth and allergy.

Methods: In a subset of 18-year-old participants from the Isle of Wight (IoW) birth cohort (n=367), the risks of birth season on allergic outcomes were estimated. Whole blood epigenome-wide DNA methylation was measured, and season-associated CpGs detected using a training-and-testing-based technique. The detected CpGs were verified in the IoW third-generation cohort of newborns and independently verified in the 8-year-old Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort. The relationships between DNA methylation, season of birth and allergy were examined.

Results: Autumn birth increased the risk of eczema, relative to spring birth. Methylation at 92 CpGs showed association with season of birth in the epigenome- wide association study. With verification, significantly more CpGs appeared to have the same directionality than expected by chance ($p=0.0067$, $\chi 2$), and four CpGs were statistically significant ($p<0.05$). Season-associated methylation was enriched among networks relating to development, the cell cycle, and apoptosis. Twenty CpGs were nominally associated with allergic outcomes. In path analyses two CpGs had marginally significant roles mediating the effect of season of birth on allergic outcomes at age 18. Season-associated methylation was largely absent in newborns, suggesting it arises postnatally due to exposures temporally associated with season of birth.

Conclusions: This study demonstrates that DNA methylation in adulthood is associated with season of birth, supporting the hypothesis that DNA methylation could mechanistically underlie the effect of season of birth on allergy, though other mechanisms are also likely to be involved.

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Environmental Protection from Asthma and Allergy: From Humans to Mice and Back

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Allergic diseases have a strong environmental component, eloquently illustrated by their increasing prevalence in the Western world over the last few decades. Among the exposures that protect from allergic disease, traditional farming has the most potent and consistent effects. Mouse studies designed to explore the mechanisms underlying allergy protection in a farming environment demonstrated that inhalation of farm dust or selected microbes can prevent allergen-driven airway hyperresponsiveness (AHR) and lung eosinophilia. However the specificity of these effects is unclear because negative controls are lacking. We chose to compare and contrast the characteristics and phenotypes of two farming populations with closely comparable genetic make-ups. The Indiana Amish and the South Dakota Hutterites are founder populations similar in their levels of inbreeding and patterns of linkage disequilibrium. Moreover, Amish and Hutterites share lifestyle and dietary factors that affect asthma and allergy susceptibility (large sibships, childhood vaccinations, diets rich in fat, salt, and raw milk, low rates of childhood obesity, long nursing durations, minimal exposure to smoking and air pollution, taboos against indoor pet). However, the Amish practice traditional farming and use animals for transportation, whereas the Hutterites embrace modern farming technologies. Strikingly, the prevalence of asthma and allergic sensitization was 5.2% and 7.2%, respectively, in Amish school children and >15% and >40%, respectively, in Hutterite school children, with profound differences in immunological profiles. As importantly, these differences could be recapitulated in mouse models. Inhalation of Amish, but not Hutterite, dust extracts was sufficient to protect ovalbumin- and house dust mite-sensitized mice from AHR and lung inflammation. Lung gene expression profiling revealed multi-pronged suppression of allergen-dependent effector pathways in mice treated with Amish but not Hutterite house dust extracts. Our results show that environmental factors underpin the opposite profiles of asthma and allergy prevalence among Amish and Hutterites. By comparing two farming environments, rather than a farming and a non-farming one, this novel approach significantly reduces the search space and lends itself to a granular dissection of the environmental and host factors involved in allergy protection.

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Maternal status in Human Pregnancy: Inherently Inflammatory or Anti-inflammatory?

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Linkages between maternal health and subsequent development of allergic phenotypes in children are complex and controversial. Although much is known of maternal-fetal interface, basal systemic innate immune status of women during and immediately following healthy pregnancies is largely unstudied. Whether a bias exists during pregnancy towards inflammatory phenotypes (transiently enhancing host defense) or anti-inflammatory phenotypes (reducing potential responses to the fetus) needs to be determined. Here, in a longitudinal study of 250 healthy women giving birth to full term healthy infants, systemic innate immune status was examined during the 2nd/3rd trimester and again one and, in a smaller subgroup, three years postpartum. Following REB approval and informed consent, pro and anti-inflammatory plasma biomarkers were quantified. Constitutive CCL2, CXCL10 and TNFa levels were sharply reduced ($p<0.003$ to 0.0001) in pregnancy. Several anti-inflammatory biomarkers were elevated (sTNFRI, sTNFRII, IL-1Ra $p<0.0001$). Plasma IL-10, evident in >85% of the population, was not altered during/post pregnancy. Kinetic studies revealed that pro-inflammatory biomarker expression (CXCL10, CCL2, CXCL8, IL-18, TNFa) was independent of gestational age. Conversely, the intensity of anti-inflammatory cytokine antagonist production increased with gestational age (Spearman $p<0.0003$). In summary, among women experiencing healthy full term pregnancies, basal systemic immunity is characterized by an increasingly intense bias towards an anti-inflammatory innate immune phenotype that is resolved by one year postpartum. Support: CRC, CIHR, AllerGen NCE

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Specific peanut allergen levels in foods: Dosing associated with oral immunotherapy and with the prevention of peanut allergy.

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Background: Innovative clinical trials of oral immunotherapy to treat current peanut allergy have been reported. The LEAP study showed that peanut consumption in early life dramatically reduced the prevalence of peanut allergy among high risk children. We investigated how the various preparations or peanut products used for therapy could be standardized to obtain realistic dose estimates for clinical trials. Specific peanut allergens in peanut butter, peanut flour and in Bamba, the preferred snack in the LEAP study, were compared. The objectives were to establish dose levels in the OIT preparations and to estimate the dose of specific peanut allergens in Bamba that could be associated with oral tolerance.

Methods: Samples (100mg) of peanut butter (n=16), peanut flour (n=11) or lots of Bamba, from either the UK (n=7) or the US (n=8), were extracted in PBS/0.05% Tween. Ara h 1, Ara h 2 and Ara h 6 were measured by ELISA using purified natural allergen standards.

Results: Peanut butter contained 2000-42,000µg/g Ara h 1 and exceeded Ara h 2 and Ara h 6 levels by 2-4fold. In contrast, peanut flours used for OIT contained 358-505µg/ml Ara h 1, 1187-5270µg/ml Arah2 and 1104-8092µg/ml Ara h 6, with Ara h 2 levels exceeding Ara h 1 by 3-10fold. Levels of peanut allergens in Bamba were remarkably consistent: Ara h 1, 2427µg/g; Ara h 2, 1970µg/g ; and Ara h 6, 2379µg/g, with 10-15% CV. The levels of each allergen in Bamba were present in ~1:1:1 ratio. Median peanut consumption in the LEAP study was 7.7g peanut per week, or 21 sticks of Bamba. This extrapolated to weekly doses of 83mg Ara h 1, 119mg Ara h 2 and 127mg Ara h 6 (total 330mg/week).

Conclusions: Variability in allergen levels in OIT extracts could affect the outcome of clinical trials of peanut OIT. Specific allergen measurements will improve standardization of peanut preparations for use in oral and transdermal immunotherapy. Bamba is a reproducible and consistent formulation of peanut allergens with the three major allergens present in uniform amounts. The results provide target doses of specific allergens that are associated with prevention of peanut allergy, which could also apply to the induction of tolerance to other food allergens.

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IgA responses to the gut microbiota in infants in relation to allergy development

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Background: The increasing allergy prevalence in affluent countries may be caused by reduced exposure and diversity of microbial stimulation, resulting in an abnormal postnatal mucosal immune maturation. While a reduced gut microbiota diversity and low mucosal total IgA levels in infancy have been associated with allergy development, IgA responses to the gut microbiota during infancy have not been studied.

Methods: The proportion of the gut microbiota bound to IgA or not were analysed by flow cytometry in faecal samples collected at 1 month and 12 months of age in 20 children developing allergy and 28 children staying healthy up to seven years of age. Furthermore, microbial composition and diversity in the sorted IgA positive and negative fractions were analysed with barcoded 16S rDNA 454-pyrosequencing.

Results: Children developing allergic manifestations, particularly asthma, during childhood had a lower proportion of IgA bound to faecal bacteria at 12 months of age than healthy children. Allergy and asthma development was also associated with reduced relative abundance of IgA coated Prevotella and Rhodococcus genera at 12 months of age. The proportion of IgA bound faecal bacteria decreased from 1 month to 12 months of age, reflecting a change from predominantly maternally breast milk derived to child derived IgA antibodies.

Conclusions: Allergy and asthma development associates with a reduced IgA responsiveness to the gut microbiota during infancy, possibly indicating an impaired mucosal barrier function.

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The role of early life risk factors for food, seasonal and perennial allergy in puberty.

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Background and aim: Allergic sensitisation (AS) is associated with allergic diseases, but recent studies suggest different disease development in subjects with no-, mono- or poly-sensitisation. Asthma with atopic dermatitis (AD) and allergic rhinitis (AR) constitute a complex phenotype with reduced lung function already from birth into adolescence. We aimed to investigate if reduced lung function or AD in infancy, gender and atopic heredity were differentially associated with type or quantity of AS in puberty.

Methods: We included all 535 children (275♂) attending 16-year follow-up investigation in the Environment and Childhood Asthma birth cohort study, 323 (164 boys) also had tidal breathing lung function (given as z-score t_{PTEF}/t_E) measured at birth. Parental atopy (asthma, AR,

AD or anaphylaxis) and socio-economic factors including family income were recorded at birth. Infant AD was recorded at 6+12 months. At 16 years AS was regarded positive with s-IgE levels ≧0.35 kU/l to any of 16 common food or seasonal/perennial inhalant allergens. The risk of AS to increasing number of allergens was assessed by Poisson regression analyses, except for food allergens (logistic regression).

Results: The majority of the 244 adolescents with AS, were poly-sensitised (2-10 allergens), (table 1). Reduced t_{PTEF}/t_E was associated with AS to food allergens and to increasing number of any allergens, but not to seasonal/perennial allergens (table 2). AS to increasing number of all allergens was higher in males (p<0.0001), parental atopy (p=0.004), reduced t_{PTEF}/t_E (p<0.0001), infant AD (p<0.0001) and higher family income (p=0.03).

Table 1. AS to number allergens	0	1	2	3 or more
Allergens				
All allergens	291 (57.1)	58 (11.4)	51 (10.0)	110 (20.6)
Food allergens n (%) Egg, milk, peanut, cod	445 (87.3)	56 (11.0)	9 (1.8)	0
Seasonal allergens n(%) Grass, birch, cladosporium, alternaria, mugwort	337 (66.5)	69 (13.6)	57 (11.2)	44 (8.7)
Perennial allergens n(%) Dog, cat, rabbit, house-dust mites, cockroach	372 (72.9)	33 (6.5)	51 (10.0)	54 (10.6)

Conclusion: Male gender was a risk factor for all types of sensitisation in puberty, whereas reduced infant lung function and infant AD appears important in food allergen sensitisation only, even during puberty, in contrast to sensitisation to seasonal or perennial allergens involving parental atopy and socio-economic factors.

TABLE 2	Sensitised to Food allergens (yes/no)*		Increasing number Seasonal allergens		Increasing number Perennial allergens	
Risk factor	Adjusted OR	95% CI	Estimate	95% CI	Estimate	95% CI
Girl	0.42	0.18 -0.92	-0.79	-1.02- -0.55	-0.56	-0.78 - -0.34
t_{PTEF}/t_E	0.63	0.39 -0.97	-	-	-	-
Infant AD	2.30	1.06 -4.95	-	-	-	-
Parental atopy	-	-	0.33	0.11 - 0.55	0.49	0.27 -0.71
Family income low-middle-high	-	-	0.21	0.08 – 0.34	-	-
* Logistic regression , 95% CI: 95 % confidence interval						

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Genetic polymorphisms of antioxidant enzymes and maculopapular eruption caused by antituberculosis drugs

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Background: Tuberculosis is still a serious health problem around the world. Of various adverse reactions to antituberculosis drugs (ATD), maculopapular eruption (MPE) is the most common cause of discontinuation of medication. While the mechanisms of ATD-induced MPE is poorly understood, oxidative stress induced by reactive drug metabolites are supposed to play an important role. Since various antioxidant enzymes protect human bodies from oxidative stress, we aimed to examine if genetic polymorphisms of antioxidant enzymes are associated with ATD-induced MPE.

Method: We enrolled 75 patients with ATD-induced MPE and 237 controls who were tolerant to the first line ATD medication including isoniazid, rifampicin, ethambutol and pyrazinamide. Genotyping was performed at single nucleotide polymorphisms in the following enzyme genes: superoxide dismutase [*SOD1* (rs2070424), *SOD2* (rs4880) and *SOD3* (rs2536512 and rs1799895)], catalase [CAT (rs1001179)] and glutathione related enzymes [*GCLM* (rs41303970), *GCLC* (rs17883901) and *GSTP1* (rs1695)]. Genotype frequencies of these SNPs were compared between case and control groups.

Results: *SOD1* gene polymorphism, rs2070424 (Ivs3-251A/G), showed significant association with ATD-induced MPE with higher frequency of genotype carrying minor allele in patients with ATD-induced MPE compared with ATD-tolerant controls (P = 0.018, OR = 2.42, 95% CI 1.16-5.05). In the other genetic polymorphisms in *SOD2*, *SOD3*, *CAT, GCLM, GCLC* and *GSTP1*, there was no significant difference in genotype frequencies between case and control group.

Conclusions: Of various antioxidant enzymes genetic polymorphisms, genetic variant in SOD1 (rs2070424) is significantly related with ATD-induced MPE. Our results suggest that SOD1 genetic polymorphism increases the risk of ATD-induced MPE.

Keywords: antituberculosis drugs, maculopapular eruption; antioxidant gene; polymorphism

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Association of Single Nucleotide Polymorphisms of Protease-Activated Receptor-2 (*F2RL1*) Gene with Asthma

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Background Proteinase-Activated Receptor-2 (PAR-2) is a G protein coupled receptor involved in inflammation that has also been implemented in asthma pathogenesis. Airway epithelium of patients with asthma or allergic rhinitis has higher PAR-2 expression compared to the epithelium of normal controls. Our lab has shown increased PAR-2 expression on peripheral blood monocytes of patients with severe asthma compared to patients with mild/moderate disease. Single nucleotide polymorphisms (SNPs) of the *F2RL1* gene, the gene name for PAR-2, have been associated with inflammatory conditions such as atopy (rs631465) and obesity (rs1529505).

Our hypothesis is that certain *F2RL1* SNPs are present preferentially in patients with severe (SA) compared with mild/moderate (MMA) asthma. We also hypothesized that PAR-2 mRNA expression in the blood is affected by the presence of these SNPs.

Methods We recruited 21 subjects with SA and 34 with MMA and collected baseline demographic and disease characteristics. We extracted genomic DNA and genotyped the two *F2RL1* SNPs mentioned above: rs1529505 (-45C/T, 5'UTR) and rs631645 (14046 C/T, coding exon). PAR-2 polymorphisms were analyzed according to asthma severity, PAR-2 mRNA in whole blood, and inflammatory markers in the blood.

Results There was no difference in the genotype and allele frequencies in the SNPs between SA and MMA. SNP rs163465 was not associated with any clinical or immunological parameters in asthmatic subjects. However, there were differences in subjects expressing the T allele of the rs1529505 SNP. These subjects had significantly higher eosinophil levels compared to other subjects in the dominant (CC vs CT+TT) and allele (C vs T) models (p=0.009, p=0.007 respectively). Patients with this polymorphism also had lower PAR-2 mRNA expression in the dominant model (p=0.012). Finally, these individuals exhibited a trend towards higher levels of mRNA expression of Th2 cell markers (p= 0.054) and higher levels of serum IL-13 (p= 0.014).

Conclusions These results suggest that SNP rs1529505 is associated with PAR-2 expression, and with markers of allergic inflammation. The mechanism of the association is not yet clear.

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Genetic variation influences the nasal microbiome

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Background: Host genetic variation has been shown to affect immune responses, but whether it can affect the airway microbiome remains unknown.

Methods: To address this question, we collected specimens from the nasal vestibule and the nasopharynx in 144 adult members of a founder population that lives communally during two seasons (summer and winter). DNA from these specimens was extracted and the 16S rRNA gene V4 region was amplified and sequenced. Bioinformatic pipelines were used to process the data and classify sequences taxonomically (QIME 1.8, Greengenes). We then assessed the effect of genetic variation on relative abundances (RAs) of bacteria identified using a linear mixed model that included sex and age as fixed effects and kinship as a random effect (as implemented in Genome-wide Efficient Mixed Model Association [GEMMA]) and available genotype data for these individuals.

Results: 332 nasal specimens were collected from 144 subjects (58 men/85 women; ages 16 to 78 years) with flocked swabs using standard protocols. 618 million sequence reads were processed and open-reference clustering was carried out at 97% identity. We subsampled 250k reads per specimen and generated relative abundances (RAs) for bacteria present in at least 75% of individuals. We found that genotype was associated with RA of a number of species at both sites and seasons. As an example, the RA of *Dermacoccus* in the summer nasal vestibule was associated with a single nucleotide polymorphism (SNP) 8kb upstream of *TINCR*, a lncRNA that binds to the peptidoglycan recognition protein 3 gene (*PGLYRP3*), which encodes a protein with known bactericidal activity (P<1.62x10-8).

For both sites and seasons, individuals with higher kinship coefficients (those who were more related) had more similar microbial beta diversity (lower UniFrac distances), indicating a correlation between genetic similarity and microbiome composition ($P<4x10^{-3}$, all). Finally, we performed pathway analysis of the genes that harbored variants associated with RA of nasal bacteria. These results indicated an enrichment of genes involved in cell to cell signaling.

Conclusions: Our findings support the hypothesis that host genetic variation can influence airway microbial composition and suggest that innate immunity and cell signaling genes may mediate such relationships. Study of the interaction between host genetics and microbiome composition may provide insights into airway biology. These studies may ultimately identify potential targets for therapeutic intervention for a range of immunologic, allergic, and respiratory diseases.

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Association of Circadian Gene Activation with Expression and Function of the Glucocorticoid Receptor in Lung Epithelial Cells

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Background: The circadian rhythm is important in both the pathogenesis and treatment of allergic airways disease but the underlying mechanisms are unknown. We aimed to investigate the relationship between the clock gene Bmal1 and the glucocorticoid receptor (GR) and the significance of these molecules in airway epithelial cell function.

Methods: Both human bronchial epithelial cells (HBE) and lung type II alveolar epithelial cells (A549) were cultured under standard conditions and were shocked with 50% horse serum for 2 hours. A549 cells were maintained in DMEM with 1% FBS serum and HBE cells were maintained in serum-free MEM supplied with growth factors. Cells were harvested for every 2 hours starting at the 6 hour time point after serum shock, for qRT-PCR, GR DNA binding activity and Western blot analysis. To assess the role of the GR rhythm, we studied mRNA expression of the immunoprotective surfactant protein D (SP-D, a glucocorticoid dependent gene) and the proinflammatory chemokine, CCL26 (eotaxin 3).

Results: qPCR showed that the expression of the clock gene BMAL1 and GR were increased in the afternoon with a peak at around 8 pm in A549 cells. BMAL1 and GR mRNA levels were positively correlated. In contrast to mRNA, GR protein levels and GR DNA binding activity to the c/EBP binding site (one of the SP-D gene promoter binding sites) were the lowest between noon and 8:30 pm in A549 cells. Similar results were observed in HBE cells. In addition, SP-D mRNA expression also oscillated over 24 hours with levels positively correlating to the BMAL1 and GR mRNA expression levels. We also found that CCL26 mRNA levels negatively correlated with the GR DNA binding activity in HBE cells implying a potential role of GR activity in controlling the proinflammatory CCL26 expression.

Conclusion: We conclude that GR and BMAL1 mRNA expression oscillated in proximal airway (HBE) and distal alveolar (A549) epithelial cells in a similar fashion. GR gene expression positively correlated with SP-D mRNA while GR DNA binding activity negatively correlated with CCL26 mRNA suggesting an important role of the circadian rhythm in immunoprotective and proinflammatory gene regulation in airway epithelial cells.

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Risk factors for COPD overlap in late-onset elderly asthma

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Background: Chronic obstructive pulmonary disease (COPD) overlap is associated with higher morbidity among asthma patients. Pathways to the development of asthma-COPD overlap syndrome (ACOS) are considered to be heterogeneous. However, risk factors have not been examined for late-onset asthma (asthma onset age ≥ 40 years old) in the elderly group (aged ≥ 65 years old).

Purpose: We aimed to examine risk factors of COPD overlap among late-onset elderly asthma patients.

Methods: We analyzed a cohort sample of late-onset elderly asthma patients (n=306) that were recruited from tertiary institutions in Korea. Spirometric value during 3-year prospective follow-up and management was utilized to define COPD overlap (FEV1/FVC<0.70) in each patient. Baseline outcomes, including demographics, smoking history, comorbidities, blood eosinophils, atopy, serum total IgE and staphylococcal enterotoxin-specific IgE (SE-IgE) levels, were analyzed in relation to COPD overlap.

Results: A total of 123 late-onset asthma patients (40.2%) were identified as having COPD overlap. Late-onset elderly asthma patients with COPD overlap had significantly older ages (72.0±5.3 vs. 70.7±4.2, p=0.020) and more smoking history (current smoker; 20.3% vs. 4.4%, p<0.001), compared to those without COPD overlap. There was no significant difference in blood eosinophil counts, atopy, or serum total IgE levels between two groups. However, those with COPD overlap had significantly higher serum SE-IgE levels than

counterparts (median 0.24 vs. 0.13 kU/L, p=0.002). In multiple logistic regression analyses, the associations of SE-IgE sensitization with COPD overlap remained significant, independently of smoking status.

Conclusions: SE-IgE sensitization, which was recently reported as the risk factor for late-onset asthma and its severity, may also contribute to the development of COPD overlap among late-onset elderly asthma patients. Staphylococcal enterotoxin could be a potential target in preventing the development of fixed airway obstruction in asthma.

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Type I and type II interferons or viral activation induce selective human mast cell production of IL-1R antagonist and VEGF-A

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Background Mast cells are abundant at mucosal surfaces where they act as sentinel cells. Interferons are critical immunoregulatory cytokines, produced in response to infection, and increasingly used to treat viral infection, immune-mediated diseases and cancers. The impact of interferon therapy on mast cells is unclear. We have characterized mediator production by human mast cells in response to interferons.

Methods Human cord blood derived mast cells were treated with recombinant recombinant type I (IFN2) and type II (IFN) interferon at a range of doses for up to 48h either in the presence or absence of pharmacological inhibitors of key signalling pathways. Mast cells were also treated with either reovirus type 3 Dearing or with Respiratory Syncytial virus. Cytokine production from mast cells was assessed by multiplex assay and by quantitative PCR analysis. The role of type 1 interferon receptors was assessed using pretreatment of mast cells with anti-IFN receptor antibodies or isotype matched controls.

Results Both IFNα2 and IFNγ induced significant ($p<0.05$) human mast cell production of the proangiogenic factor VEGF-A and IL-1 receptor antagonist in addition to IL-17, CXCL10 and CCL5. CXCL10, VEGF-A and IL-1Ra were selectively induced in the absence of degranulation in a dose and time dependent manner via classical JAK-STAT signaling. When mast cells were infected with reovirus or respiratory syncytial virus (RSV). Mast cells secreted VEGF-A, IL-1Ra and IFNα2, but not IFNγ, in response to either reovirus or RSV activation. RSV-induced IL-1Ra, but not VEGF production, was dependent on type I IFN receptor signaling.

Conclusions These findings support an important role for mast cells in the immunoregulatory and tissue remodelling responses to interferons used therapeutically and in multiple interferon-rich mucosal environments including sites of viral infection.

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Mast cells are critical for the healing of infected wounds in mice

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Background: Skin wound infection is a considerable health problem that gains importance due to increasing antibiotic resistance. A better understanding of the innate defense mechanisms against bacterial superinfection could lead to novel treatment approaches. Mast cells (MCs) have been shown to contribute to optimal host defense against bacterial infections. However, the role of MCs in bacterial infections in a clinical relevant model of skin wound infection is poorly understood.

Methods: We therefore established a model of *Pseudomonas aeruginosa* (PA) skin wound infection in mice and characterized the critical factors involved in optimal wound healing using $Kit^{W}/Kit^{W\text{-}v}$ and Cpa3-Cre; Mcl-$1^{fl/fl}$ MC-deficient mice, MC-deficient mice engrafted locally with WT MCs or IL-6-deficient MCs and topical recombinant IL-6 treatment.

Results: We observed significantly delayed wound closure in PA infected skin wounds in the absence of MCs in $Kit^{W}/Kit^{W\text{-}v}$ and Cpa3-Cre; Mcl-$1^{fl/fl}$ MC-deficient mice. This delay in wound closure was associated with a 10-fold reduction in bacterial clearance. Engraftment of MCs into the skin of $Kit^{W}/Kit^{W\text{-}v}$ mice restored both, bacterial clearance and wound closure, to wild type levels. Co-culture of MCs and keratinocytes (KCs) infected with PA led to a significant increase of MC-derived IL-6 *in vitro* and local engraftment of MC-deficient $Kit^{W}/Kit^{W\text{-}v}$ mice with IL-6-deficient MCs failed to control PA wound infection and restoration of normal wound healing *in vivo*. Treatment with recombinant IL-6 was able to induce antimicrobial peptide production by KCs *in vitro* and resulted in the control of PA infection and normal wound healing *in vivo*.

Conclusions: Taken together, our results demonstrate that skin wound infection by PA is controlled by MCs and reveal a novel antimicrobial defense mechanism that requires the release of MC-derived IL-6. These findings offer new strategies for the prevention and treatment of antibiotic resistant bacterial infections.

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Elimination of human lung mast cells in situ by the anti-malarial drug mefloquine: a new principle for targeting mast cells in asthma?

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Mast cells are currently emerging as major candidates for therapeutic intervention in allergic asthma, because they are responsible for many of the features associated with the disease. Therefore, strategies that limit lung mast cell-mediated functions can offer an attractive treatment option for asthmatic patients. Limiting mast cell harmful functions can be accomplished by several ways, including prevention of mast cells degranulation or by targeting of individual products secreted by mast cells. However, an even more efficient way to block mast cell activities would be to eliminate the detrimental mast cell populations locally, for instance through induction of apoptosis. Here we tested if mefloquine, which is a lysosomotropic agent, can induce apoptosis of lung mast cells. The rationale behind this approach is that mast cell are exceptionally rich in lysosome-like granules, and thus lysosome permeabilization will lead to the release of granule-localized compounds including proteases into the cytosol where they potentially may induce apoptosis. Based on this principle, we demonstrate for the first time that incubation of human lung tissue biopsies (obtained from patients undergoing surgery for lung cancer) with mefloquine markedly reduced mast cell populations, with no adverse effect on the lung morphology. Double-staining of the lung tissue sections with TUNEL and tryptase (a mast cell-specific protease) revealed that the human lung mast cells underwent apoptotic cell death rather then necrosis in response to mefloquine. Apoptotic cell death was also confirmed when performing annexin V/PI staining of mefloquine-treated purified primary lung mast cells. These findings raise the possibility of using lysosomotropic agents as a novel approach to ablate harmful lung mast cell populations in mast cell-driven disease such as allergic asthma.

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Combining micro-computed tomography and immunohistochemistry to visualise mast cell 3D distribution in Idiopathic Pulmonary Fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF) is the most common interstitial lung disease with more than 5000 new cases in the UK per annum. Yet its pathology remains poorly understood. It is a chronic, fibrotic lung disease with increased mast cell numbers however the contribution of mast cells to the pathophysiology and fibrosis in IPF is unknown.

Aims: To investigate relationships between mast cells and structural elements of lung in IPF using a combination of micro-CT, conventional histology and immunohistochemistry.

Methods: A formalin fixed, paraffin embedded lung biopsy from a patient with IPF was non-destructively scanned using micro-CT and reconstructed with a resolution of 8µm. The biopsy was then sectioned and slides were stained at regular intervals using Movat's pentachrome. A random section was immunostained with AA1 antibody against mast cell tryptase. Blood vessels and airways were semi-automatically segmented and mast cells were manually segmented with reference to immunostaining, allowing them to be visualized in 3 dimensions in the microCT volume.

Results: The positions of 21767 mast cells were successfully added to the microCT dataset of the IPF tissue. Mast cells were completely absent from areas of established fibrosis but were identified in surrounding areas. Vasculature and airways were successfully segmented revealing their complex inter-relationships. We were then able to analyse the relationship between airways, blood vessels, the pleural surface and the mast cells in 3D. Using 3D distance maps, distances between large numbers of mast cells and other segmented structures were measured and analyzed.

Conclusion: IPF lung tissue contains substantial numbers of mast cells and by combining micro-CT with conventional histology and immunohistochemistry the distribution of mast cells in the lung can be extended to 3 dimensions.

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Papain activates human mast cells to release pro-inflammatory mediators via its enzymatic activity

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Mast cells (MCs) IgE-independent activation is an important reaction that can take place even in an allergy setting due to endogenous substances such as NGF and MBP-1 secreted from eosinophils and/or other mediators secreted from different cells in late and chronic stages of allergic inflammation. Also it can be elicited by allergens such as by the protease derp1, derf1, pera10 and others probably

even at the first exposure. Papain a food derivative and a cosmetic component, is an allergen belonging to the cysteine proteases. It is particularly interesting since it can induce itch without evident histamine release, impairs the skin barrier and increases MCs number. In the present work we have hypothesized that papain could induce an IgE-independent MCs activation.

Papain added to foreskin pieces (1hr) caused MC activation as detected by toluidine blue staining and by tryptase release. Papain also caused a dose-dependent activation of purified foreskin MCs and of human cord blood derived MCs (CBMC) as shown by β-hexosaminidase release (1h) at a later time point (3-6hrs) of IL-8 and IL-6. Next we addressed the question of whether the papain-induced MC degranulation is due to its specific protease activity. Therefore before its addition to CBMC, papain was heat-inactivated (10 min at 100°C), or preincubated with the cysteine protease inhibitor E-64. Both treatments abrogated papain mediated MC degranulation at a similar extent. To check whether papain effects on MCs are mediated by PAR-2, CBMC were pretreated with the specific PAR-2 antagonist ENMD-1068 before incubation with papain. This treatment partially inhibited CBMC degranulation. When CBMC were preincubated with the Gi protein inhibitor benzalkonium chloride and then exposed to papain, robust inhibition of papain mediated CBMC activation was observed. Furthermore papain was shown to induce phosphorylation of Lyn.

In conclusion, this study demonstrated that papain is a direct activator of human MCs via PAR-2 and its enzymatic activity. Papain activation modalities can be shared by other protease allergens. The MCs dual activation, by IgE dependent and IgE independent mechansims, could be much stronger than the one brought about by non-proteases allergens and could need two different prophylactic approaches.

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Statins Suppress Mast Cell Function and Survival

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Mast cell- and basophil-associated inflammatory diseases are a considerable burden to society. Statins, used to lower serum cholesterol, have immune modulating activities. We tested the in vitro and in vivo effects of statins on IgE-mediated mast cell and basophil activation. Fluvastatin showed the most significant inhibitory effects of the six statins tested, suppressing IgE-induced cytokine secretion among mouse mast cells and basophils. Fluvastatin effects were reversed by mevalonic acid or geranylgeranyl pyrophosphate, and mimicked by geranylgeranyl transferase inhibition. Fluvastatin selectively suppressed key FcεRI signaling pathways, including Akt and ERK. While mast cells and basophils from the C57BL/6J mouse strain were responsive to fluvastatin, those from 129/SvImJ mice were completely resistant. Resistance correlated with fluvastatin-induced upregulation of the statin target hydroxymethylglutaryl (HMG)-CoA reductase. Human mast cell cultures from eight donors showed a wide range of fluvastatin responsiveness. Finally, exposure to fluvastatin for more than 48 hours induced apoptosis in mouse mast cells that correlated with loss of mitochondrial membrane potential. These data demonstrate that fluvastatin is a potent suppressor of mast cell function and survival acting at least partly via blockade of geranyl lipid production downstream of HMG-CoA reductase. Importantly, consideration of statin use for treating mast cell-associated disease needs to incorporate genetic background effects, which can yield drug resistance.

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Mechanisms of eosinophil degranulation and impact on airway responses in allergic asthma

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Background: Eosinophils are prominent pro-inflammatory cells in allergic inflammation, and are elevated in allergic asthmatics with eosinophilia. Degranulation from eosinophils is considered to be a key event in allergic inflammation. Here we investigate the roles of intracellular signalling molecules in the regulation of eosinophil degranulation and how this contributes to allergic airway responses. Our preliminary findings demonstrated that the Rab GTPase Rab27a, as well as the SNARE molecule VAMP-7, regulate the release of eosinophil-derived mediators, eosinophil peroxidase, eosinophil-derived neurotoxin, and major basic protein.

Hypothesis: Degranulation in eosinophils is regulated by Rab27a and VAMP-7, these contribute to the development of airway responses in allergic asthma.

Methods: To test this hypothesis, we examined Rab27a knockout mice (*ashen*) for their responses to ovalbumin as a model of allergic airway inflammation. We also generated an eosinophil-specific gene knockout mouse of VAMP-7. This was carried out with *eoCre* mice crossed with floxed VAMP-7 mice to generate *eoCre-V7* mice that lack VAMP-7 only in eosinophils. *Ashen* and *eoCre-V7* mice were crossed with IL-5 transgenic mice to generate sufficient numbers of eosinophils to determine their effects on the release of eosinophil granule proteins as well as cytokines and chemokines.

Results: Rab27a-deficient eosinophils from *ashen* mice crossed with IL-5 transgenic mice showed reduced degranulation responses. In correlation with this, *ashen* mice showed diminished airway hyperresponsiveness to methacholine challenge. Eosinophil-specific knockout of VAMP-7 also showed diminished eosinophil degranulation as well as reduced airway hyperresponsiveness in ovalbumin-sensitized and challenged mice.

Conclusions: These data suggest that Rab27a and VAMP-7 contribute to eosinophil degranulation. While Rab27a contributes to allergen-induced airway responses, it could not be determined if this was related to eosinophil degranulation. In contrast, VAMP-7-dependent eosinophil degranulation may have a role in the promotion of allergen-induced airway responses.

Funded by: Canadian Institutes of Health Research.

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Morphological grading of bone marrow biopsy for the diagnosis mast cell activation syndrome.

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Introduction: The diagnosis of mast cell activation disorder currently relies on a consensus of clinical criteria. Even so, diagnosis remains troublesome in some patients and differentiating between related disease such as idiopathic anaphylaxis can be difficult.

Methods: Archived bone marrow (BM) samples from all patients since 2005 suspected of a mast cell disorder that were not diagnosed with mastocytosis were retrospectively reviewed and scored on 15 different morphological criteria blinded to the diagnosis. In addition, a small cohort of random BM samples of ISM patients was analyzed. Patterns were analyzed in a training sample and the performance of this scoring system was measured in a separate subgroup. The training sample consisted of 50 random BM samples of whom ten were diagnosed with idiopathic anaphylaxis (IA), 4 with cutaneous nastocytosis (CM), 4 indolent systemic mastocytosis (ISM) ,3 monoclonal mast cell activation syndrome (MMAS), 12 mast cell activation syndrome (MCAS) and 18 with other varying diagnoses ranging from food and environmental allergy to hematological malignancies. Patterns were analyzed using principal component analysis (PCA) with oblimin rotation based on an eigenfactor >1. The identified patterns were used to make a scoring system using reciever operater characteristics curves valuing specificity 50% more than sensitivity.

Results: PCA revealed a similar underlying component for patients with CM, ISM, MMAS and MCAS, but not with IA. This component was defined by a higher mast cell %, more spindle shaped mast cells, larger mast cells, the occurrence of small aggregates, and in the case of MCAS, notable eosinophilia. We grouped these diseases together under the name "mast cell disorders". The only other disease classification showing occasionally similar bone marrow morphology were unrelated hematological malignancies. A scoring system, the Boston Mast Cell Disorder Score (BMCD-score) was devised based on this pattern and tested in a group consisting of the training set plus 50 other BM biopsies. After exclusion of unrelated hematological malignancies, the BMCD-score resulted in a sensitivity of 58% and a specificity of 91%.

Conclusion: Bone marrow morphology in MCAS shares common features with mastocytosis. BMC-score was highly specific in differentiating between mast cell disorders such as MCAS and IA.

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Prognostic And Predictive Significance Of Tumor Associated Neutrophils In Colorectal Cancer

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Background: Neutrophils play a key role in the acute phase of inflammation and resistance against pathogens. Recent evidence placed neutrophils as key effector cells in the orchestration of the innate and adaptive immune responses. However, the occurrence and significance of tumor-associated neutrophils (TAN) and their roles in cancer have been the subject of conflicting reports.

Objective: Our objectives were to describe a reliable method to assess TAN infiltration in colorectal cancer (CRC), to evaluate their clinical significance and to assess their roles in cancer.

Method: CRC tissues (n=271) (stage I to stage IV) were retrospectively investigated by immunohistochemistry (CD66b) on whole tumor sections. $CD66b^+$ immunoreactive area (IRA) was measured by using a computer-assisted image analysis system in three contiguous but not overlapping fields both at the invasive tumor margin (IM) and in the intratumoral compartment (IT). In addition, freshly isolated human neutrophils were stimulated, in *vitro,* with conditioned media from colorectal cancer cell lines (SW480, HT29, SW620).

Results: In Stage I-Stage IV CRC patients, higher IT and IM CD66b+ IRA was associated with better Disease Specific Survival (DSS) (p=0.002 and p=0.003) and better Disease Free Survival (DFS) (p=0.03 and p=0.03). Multivariate Cox proportional hazard analysis showed that high IT CD66b+ IRA was an independent prognostic factor for better DSS (HR= 0.48; p= 0.03) and better DFS (HR=0.56;

p=0.05). IT CD66b+IRA was also found to be predictive for 5-FU chemotherapy response in Stage III CRCs (n=178, p=0.01). Finally, CRC cell lines produced soluble factors promoting neutrophil chemotaxis and survival. In turns, neutrophils displayed cytostatic activity against CRC cell lines.

Conclusions: Our findings showed that TAN infiltration was associated with good prognosis and response to 5-FU based chemotherapy in CRC patients. In addition, we found that CRC cell lines produce soluble factors able to 're-educate' neutrophils towards an anti-tumoral functional state. These data will advance our understanding on the molecular and cellular mechanism of cancer-related immune response and will pave the way for new therapeutic approaches.

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Cellular uptake and degradation of allergens inside dendritic cells.

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Background: Cellular uptake and degradation of proteins in critical immune cells such as dendritic cells (DC) is crucial for the induction of adaptive immune responses. However, it is unknown how this process defines allergic sensitization. Since industrial processing influences protein and allergen structure, size and activity, we hypothesize that biochemical processing of allergens affects their cellular uptake and degradation and hereby affects the development of allergy.

Method: In our studies, we studied the cellular uptake and degradation in DC of the important milk allergen ß-lactoglobulin, before and after relevant processing. For this purpose, ß-lactoglobulin was heated and/or glycated in the presence of different sugars. Hereafter, the uptake and degradation of these preparations in mouse bone marrow-derived DC was determined. Several pathways involved in the uptake of proteins were inhibited by specific inhibitors.

Results: Heating and glycation changed the secondary structure and led to the aggregation of ß-lactoglobulin. Interestingly, this led to the increased cellular uptake compared to native protein. In addition, heating and glycation enhanced the intracellular degradation of ß-lactoglobulin in DC. The uptake of all ß-lactoglobulin preparations was mediated via receptor-mediated endocytosis and micropinocytosis, whereas the uptake of heated and glycated ß-lactoglobulin was also partly mediated via phagocytosis. Finally, maturation of DC drastically reduced the uptake and degradation of all forms of ß-lactoglobulin.

Conclusions: Modification of a known well-characterized allergen led to significant changes in the uptake and degradation of this allergen and led to differences in cellular pathways of uptake. This may lead to differences in the allergenicity of novel or modified proteins via antigen presentation, which is addressed by current studies. In conclusion, we demonstrated the usefulness of DC-based assays to examine initial cellular responses to potential novel or modified allergens.

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Clusterin Modulates Allergic Airway Inflammation by Attenuating CCL20-mediated Dendritic Cell Recruitment

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Background: Recruitment and activation of dendritic cells (DCs) in the lungs is critical for Th2 responses in asthma. CCL20 secreted from bronchial epithelial cells (BECs) influences the recruitment of DCs. This process is suggested to be associated with oxidative stress regulation. Clusterin, an oxidative stress regulatory molecule, may play a pivotal role in the pathogenesis of allergic airway inflammation. The aim of this study was to examine if clusterin functions in the regulation of CCL20 production from BECs and is involved in allergic airway inflammation through DC recruitment.

Method: Clusterin knockout (KO) mice were exposed to house dust mite (HDM) extract to induce allergic airway inflammation. We observed the effect of clusterin on production of CCL20, development of allergic airway inflammation, immune cell recruitment to the lung, and intracellular production of reactive oxygen species (ROS). CCL20 and ROS generation was then evaluated in HDM-stimulated human BECs in the setting of clusterin overexpression and downregulation.

Results: The number of total immune cells in bronchoalveolar lavage fluid (BALF) and the lung were dramatically increased in clusterin KO mice. Inflammatory DC ($CD11b^+CD11c^+$) and neutrophil populations in the lung were also significantly increased, which was

accompanied by increased CCL20 expression in BALF and oxidative stress marker expression in the lung. HDM-stimulated clusterin up- and downregulated BECs showed that CCL20 secretion was negatively correlated with clusterin expression. Clusterin attenuated intracellular ROS production, which is related to induction of CCL20 expression after HDM stimulation.

Conclusions: Thus, clusterin may modulate recruitment of DCs to the airway by regulating CCL20 production.

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Vitamin D3 down regulates the high-affinity receptor for IgE (FcepsilonRI) on human dendritic cells.

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Background: The expression of FcepsilonRI on epidermal dendritic cells (DC) is a hallmark for atopic dermatitis (AD) and its density correlates with the severity of the disease. We showed previously that TLR2 engagement reduces FcepsilonRI expression on the transcriptional level, mediated by PU.1 and accompanied by maturation. Vitamin D3 is synthesized in the skin and AD severity and IgE level are reported to correlate with vitamin D3 level in the patients' serum. We hypothesize that vitamin D3 may influence the expression of FcepsilonRI receptor on DC.

Materials and Methods: We investigated DC from different origins: (i) immature Langerhans cells (LC) were generated from $CD34^+$ hematopoietic stem cells (CD34LC), (ii) immature DC were generated from monocytes (moDC) and (iii) LC were prepared from skin of healthy donors and AD patients. These different DCs were stimulated with active vitamin D3 (calcitriol). Surface expression of FcepsilonRI and maturation marker CD83 were analyzed by FACS while mRNA encoding the receptor components FCER1A, FCER1G and its transcription factors (TF) were analyzed using real-time PCR.

Results: We observed that CD34LC treated with calcitriol down-regulated FcepsilonRI expression dose-dependently while CD83 expression was not altered. FCER1A mRNA was reduced as well suggesting a regulation on the transcriptional level. FCER1G was little affected. Contrary to TLR2-mediated regulation, analysis of TF showed no down-regulation of PU.1 by calcitriol and PU.1-related microRNA-155 was unaffected. TF YY1 and ELF1 were little up-regulated and HMGB1 and HMGB2 were significantly increased, but were regulated at later time points than FCER1A. Preliminary data show that moDC and skin LC expressing FcepsilonRI were susceptible to vitamin D3-mediated down-regulation.

Conclusion: Thus, vitamin D3 can down-regulate FcepsilonRI in different DC subsets indicating a general mechanism most likely on transcriptional level. Vitamin D3 uses a different mechanism than TLR2 for FcepsilonRI regulation which remains to be elucidated. Regulation of FcepsilonRI by vitamin D3 might be important for allergen-specific immune mechanisms in AD.

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Evidence that basophil-derived tumor necrosis factor can enhance certain innate or adaptive immune responses in mice

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Background: Tumor necrosis factor (TNF) is critical for mounting effective immune responses against certain pathogens, but in excess it can contribute to excessive inflammation and sepsis. The identification of cellular sources of TNF is important for understanding the mechanisms that regulate TNF production in health and disease. In this study, we investigated whether basophil-derived TNF can significantly contribute to inflammation during selected innate and adaptive immune responses.

Methods and Results: We generated a conditional mouse in which TNF production was specifically ablated in basophils (*Basoph-8-Cre; Tnf$^{fl/fl}$* mice) by crossing mice that transgenically expressed *Basoph-8*-Cre with mice containing loxP-flanked *Tnf* alleles. Numbers of dermal leukocytes in the ear pinnae of the *Basoph-8-Cre; Tnf$^{fl/fl}$* mice were reduced at sites of IgE- and basophil-dependent chronic allergic inflammation (CAI), suggesting that local release of basophil-derived TNF contributes to leukocyte recruitment after CAI. Serum and intraperitoneal TNF levels were significantly reduced after i.p. challenge with a sub-lethal dose of LPS, indicating that basophils contribute to increased TNF levels during LPS-induced endotoxemia. Basophil-derived TNF also may contribute to neutrophil migration from the blood and recruitment into tissues through up-regulation of CD11b expression after LPS challenge. Finally, basophil-derived TNF also significantly contributed to mouse survival the cecal ligation and puncture (CLP) model of experimental sepsis, probably by enhancing the ability of neutrophils and macrophages to clear bacteria.

Conclusions: Our findings in *Basoph-8-Cre; Tnf*$^{fl/fl}$ mice provide strong evidence that basophil-derived TNF can contribute importantly to inflammation in certain adaptive and innate immune responses.

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Role of costimulatory signals in the induction of T cell steroid resistance in asthma

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Background: To investigate the role of helper T (Th) cells in severe asthma, steroid responsiveness of Th cells was analyzed.

Method: PBMC obtained from mild (steroid sensitive, SS), steroid dependent (SD), and steroid resistant (SR) asthmatics were stimulated with mitogens. Der f 2-specific Th clones were established and effects of glucocorticoids (GCs) on the proliferation and cytokine synthesis were analyzed. Steroid responsiveness of murine Th clones reactive to ovalbumin were analyzed. Unprimed BALB/c mice were transferred with these clones and then antigen challenged. Effect of GC on BALF eosinophilia was evaluated. CTLA4-Ig was administered through nasal inhalation or venous injection.

Result: IL-5 production by PBMC of SS asthmatics was significantly reduced after ICS administration, but that of SD asthma remained high. IC_{50} values for the suppression of cytokine synthesis and proliferation responses by dexamethasone was not different among SS, SD, or SR asthmatics. Addition of CD28 signal induced steroid resistance in IL-2 and PI-3 kinase dependent manner. Murine SS and SR Th clones were selected based on the steroid sensitivity of their proliferation responses *in vitro*. Airway infiltration of eosinophils and lymphocytes of mice transferred with SS clones were effectively inhibited by GC. In contrast, those of mice transferred with SR clones were not significantly inhibited. Administration of CTLA4-Ig significantly suppressed the proliferation of GC-treated SR clones *in vitro*, and the BALF eosinophilia of mice transferred with SR clones *in vivo*.

Conclusion: Costimulatory signal mediated through CD28 seems crucial for the induction of steroid resistance and might be a target for therapeutic intervention.

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IL-4 abrogates T(H)17 cell-mediated inflammation by selective silencing of IL-23 in antigen-presenting cells

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Interleukin 4 (IL-4) can suppress delayed-type hypersensitivity reactions (DTHRs), including organ-specific autoimmune diseases in mice and humans. Despite the broadly documented antiinflammatory effect of IL-4, the underlying mode of action remains incompletely understood, as IL-4 also promotes IL-12 production by dendritic cells (DCs) and IFN-γ-producing T(H)1 cells in vivo. Studying the impact of IL-4 on the polarization of human and mouse DCs, we found that IL-4 exerts opposing effects on the production of either IL-12 or IL-23. While promoting IL-12-producing capacity of DCs, IL-4 completely abrogates IL-23. Bone marrow chimeras proved that IL-4-mediated suppression of DTHRs relies on the signal transducer and activator of transcription 6 (STAT6)-dependent abrogation of IL-23 in antigen-presenting cells. Moreover, IL-4 therapy attenuated DTHRs by STAT6- and activating transcription factor 3 (ATF3)-dependent suppression of the IL-23/T(H)17 responses despite simultaneous enhancement of IL-12/TH1 responses. As IL-4 therapy also improves psoriasis in humans and suppresses IL-23/T(H)17 responses without blocking IL-12/T(H)1, selective IL-4-mediated IL-23/T(H)17 silencing is promising as treatment against harmful inflammation, while sparing the IL-12-dependent T(H)1 responses.

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Platelets influence B cells via CXCL12

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Background: Although the main function of platelets is to prevent bleeding, they can also influence innate and adaptive immune responses. When investigating the immediate effects of splenectomy on peripheral blood B cells, downregulation of B cell surface CXCR4 (chemokine receptor 4) was observed within the first week of the procedure, which returned back to baseline at 3 weeks. The initial decrease in CXCR4 expression coincided with the transient thrombocytosis that follows splenectomy. We hypothesized that platelets induced the down-regulation of CXCR4 via Stromal cell-derived factor 1a (SDF-1a) also known as chemokine ligand 12 (CXCL12).

Methods: We co-cultured platelets in sterile tubes containing 10^6 PBMC (peripheral blood mononuclear cells) in 2% PBS to give a final volume of 100 uL. Platelets and cells were harvested at varying time points. After removal from cell culture the platelets and cells were stained with conjugated antibodies and fixed with paraformaldehyde. After addition of fixative the samples were processed by flow cytometry. B cells were defined as CD45+/CD20+ and platelets were defined as CD41+/CD61+.

Results: First, to demonstrate that platelets secrete SDF-1a, intracellular staining was performed before and after stimulation with PMA **(Phorbol myristate acetate).** At baseline, 25% of platelets expressed SDF-1a and this increased to 60% upon stimulation. We then incubated varying numbers of platelets from 500,000 platelets/mcl up to 2 million platelets/mcl with PBMC (peripheral blood mononuclear cells) and stained for CXCR4 on B cell surface at 0, 4, 6, 8, 12 and 24 hours. At 6 hours CXCR4 expression increased, followed by a down-regulation at 12 and 24-hours. This effect was partially blocked by the addition of anti-SDF1, and possibly due to other cytokines working through CXCR4 such as MIF1 (macrophage migration inhibiting factor).

We then looked at platelets from patients with primary anti-phospholipid syndrome (aPS) PMA stimulation resulted in almost 100% of platelets to express SDF1. Hence, platelets may utilize the CXCL12/CXCR4 pathway not just to attract B cells to sites of inflammation but also influence auto-antibody generation as seen in aPS patients. Further investigations in platelet B cell interaction should provide useful insights into inflammatory and autoimmune disorders.

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Cellular crosstalk between airway epithelial and endothelial cells augments CX3CL1 shedding during viral infections

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Introduction: The epithelial and endothelial barriers of the airway mucosa are critical for regulation of tissue homeostasis and protection against pathogens and other tissue damaging agents. In response to a viral infection, epithelial cells must signal to the endothelium to initiate immune cell recruitment, however the mechanisms of this cross-talk are not fully understood. CX3CL1 (fractalkine) is a membrane-bound chemokine expressed mainly on epithelial and endothelial cells, and can be shed as a soluble chemotactic factor. In asthma, epithelial CX3CL1 expression is increased and CX3CL1 expression is responsive to rhinovirus infection *in vitro*. Since respiratory viral infections are a major cause of asthma exacerbations, we postulated that viral infections can trigger epithelial-endothelial crosstalk involving CX3CL1.

Methods: We developed a microfluidic 3D co-culture model of polarised airway epithelial cells (AECs) and endothelial cells to analyse the kinetics of CX3CL1 release and identify cytokine(s) potentially involved in cell-cell cross talk. The 3D model was challenged apically with double stranded RNA (dsRNA, Polyinosinic-polycytidylic acid), a molecular pattern associated with viral infection. Basal supernatants were collected at 2h intervals and mediator secretion determined by ELISA.

Results: In contrast to static cultures, the microfluidic system allowed time-dependent analysis of airway barrier responses at shorter intervals and with greater sensitivity. Using this system, challenge of the apical epithelial surface with dsRNA induced release of CX3CL1 which was synergistically enhanced in epithelial-endothelial co-cultures *versus* epithelial or endothelial monocultures. CX3CL1 release was preceeded by an increase in TNF-α which occurred equivalently in epithelial monocultures or epithelial-endothelial co-cultures, suggesting that the epithelium is the primary source of this cytokine. A role for TNFα in driving CX3CL1 release was confirmed by neutralisation of TNFα. Endothelial cells within the co-culture also responded to dsRNA with upregulation of ICAM-1 and E-selectin in a TNFα-dependent manner.

Conclusion: During viral infections, endothelial-derived CX3CL1 release and adhesion molecule expression is triggered by epithelial derived TNF-α. This cellular crosstalk might play an important role in the co-ordinate recruitment of CX3CR1 expressing immune cells such as $CD4^+$ T cells, monocytes, macrophages, cytotoxic T cells and natural killer (NK) cells into the airways during a virus-induced asthma exacerbation.

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Activating Transcription Factor 3 (ATF3) causes susceptibility to opportunistic infections during post-septic immunosuppression

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Postseptic immunosuppression, also known as compensatory anti-inflammatory response syndrome (CARS), causes most sepsis-related deaths. Yet, the molecular mechanisms underlying this phenomenon are elusive as it paralysis all immune functions. Analysing blood samples of humans during CARS, we found a significant and close correlation of severely suppressed glutathione-levels with the strong induction of ATF3 (activating transcription factor 3) and the loss of activation induced IL-6. ATF3 is the first transcription factor

in the NF-κB signalling pathway induced after innate immune stimulation. Thereby, ATF3 negatively regulates the transcription of IL-6 and TNF. As IL-6 and TNF are key cytokines involved in the antimicrobial defence, we speculated that ATF3 might be a key transcription factor responsible for the postseptic immune suppression and the increased susceptibility to opportunistic infections. To test this hypothesis we used CLP (cecal ligation and puncture), one of the best-established models of bacterial sepsis. We first induced sublethal CLP in wild type (wt) and ATF3$^{-/-}$ mice, to closely imitate the clinical conditions in mice. Subsequently, we challenged mice during the postseptic CARS with the fungal pathogen *Aspergillus fumigatus,* at doses that are non-pathogenic to healthy mice. Post-septic wt-mice rapidly succumbed to this sub-lethal pulmonary *Aspergillus fumigatus* infection. In sharp contrast, ATF3$^{-/-}$ mice had not only a significantly prolonged survival, 20% of these mice even survived this infection that was lethal in 100% of wt mice. Thus, ATF3 is the first transcription factor identified that determines susceptibility to and the course of opportunistic infections.

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Lymphoproliferative Disease in the setting of STK4 defect

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Background: STK4 encodes the Mammalian Ste20-like Kinase (MST) 1, which functions largely as regulators of proliferation, apoptosis, morphology, and cell shape. STK4 defects in humans are rare and can lead to recurrent infections, lymphopenia, and neutropenia, but generally affecting the innate immune system. We present a 14-year-old boy with lymphoproliferative disease, who was found to have a defect in the STK4 gene. He initially presented to the emergency room with acute abdominal pain, and was found to have enlarged intra-abdominal lymph nodes. He had no history of recurrent fevers or weight loss. His EBV, CMV and Adenovirus PCR in peripheral blood were negative. He had no other lymphadenopathy or organomegaly on exam. His past medical history was significant for renal failure due to hemolytic uremic syndrome at 6 years of age. There was no history for recurrent infections or any other autoimmune disease. Family history was noncontributory. He had exploratory laparotomy and lymph node resection. Histological exam of the lymph nodes did not reveal any malignant cells.

Method and Results: Immunostaining of the lymph node showed no nodular hyperplasia and regressed germinal centers with lack of secondary B cell follicles and plasma cells. B cells in primary follicles lacked IgM expression. There were very few CD45RO+ T memory cells and most expressed very high programmed death-ligand 1 (PD-L1). A genetic work-up was pursued due to multiple dysmorphic features, hearing loss and borderline intellectual disability. Chromosome analysis was normal but Whole Exome Sequencing (WES) revealed a genetic change in the STK4 gene at Arg310Gly with a prediction of protein function being affected. Peripheral blood immunophenotyping showed normal T, B and NK cell counts, normal central (CCR7+CD45RO+) and effector (CCR7-CD45RO+) memory T-helper and T killer cells but decrease in IgG expressing B cells. Further analysis of his T cells showed normal proliferation and interferon gamma production. His neutrophil showed significant reduction in the expression of surface CD11b and CD16.

Conclusion: The immunological findings in this case suggest B cell and neutrophil defect with preserved T cell function and raise the possibility of STK gene in adaptive immunity.

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Lipid mediators in asthma: Defining targets and biomarkers in severe asthma cohorts and experimental medicine studies

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Background: Anti-leukotrienes are the first targeted treatment of asthma, but their use and relative effectiveness remains under debate. Likewise, the roles of prostaglandins (PG) and other cyclo-oxygenase (COX) products in asthma remain unclear, not the least due to presence of the clinical syndrome of aspirin/NSAID-intolerant asthma. Since the previous CIA meeting we have however gathered a lot of data that fill some of the knowledge-gaps:

Methods & Results: 1) Using a new UPLC-MS/MS platform for analysis of urinary eicosanoid metabolites in the European U-BIOPRED cohort of 600 adult asthmatics and healthy controls, most pathways including the oxidative stress markers isoprostanes, were exaggerated in severe asthma. Stratifying for high urinary LTE4 identified a Th2- type sub-phenotype with high sputum and blood eosinophils, high urinary PGD2 metabolites, high total IgE and exhaled nitric oxide (FENO), as well as high circulating IL-13. The data were validated in the BIOAIR cohort (n=250) of severe and controlled asthma where a 2 wk controlled oral prednisone trial did not change urinary eicosanoid metabolites.

2) In the isolated human small bronchi, PGE2 displayed the anti-asthmatic property to blunt mast cell- mediated bronchoconstriction by an EP2 driven inhibition of the release of histamine and CysLTs. We furthermore established a new in vitro method to define the mechanisms of exercise-induced bronchoconstriction by challenge of the isolated human bronchi with hyperosmolar mannitol which caused a mast cell-dependent contraction. The prostanoids PGD2, PGF2α & TXA2 all contracted human bronchi solely by activation of the TP receptor.

3) The bronchoconstriction and sputum eosinophilia produced by inhalation of LTE4 was blocked by the CysLT1 antagonist montelukast in a controlled study of 13 subjects with asthma.

Conclusions: Lipid mediator pathways remain central to the pathophysiology of asthma with urinary LTE4 and PGD- metabolites being new predictive non-invasive biomarkers of the Th2 profile. Proper use of anti- leukotrienes for treatment of asthma and allergic conditions need to consider them as part of the mast cell activation cascade. Complete blockade of the main mast cell prostanoid PGD2 requires combined TP antagonism and CRTH2 antagonism, whereas there seems to be no role for the murine E-type receptor for LTE4 in human airways. Finally, EP2-receptor agonism has potential as a new treatment for asthma and allergies.

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Hemopoietic progenitor expression of epithelial cytokine receptors in allergic asthma: role of Toll-like receptors

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Background: In previous work, we demonstrated that cord blood (CB) human hemopoietic progenitor cells (HPC) from high allergic risk newborns express toll-like receptors (TLR) less abundantly compared to those of low risk. We also have recently found a key role for thymic stromal lymphopoietin (TSLP) in human eosinophil and basophil (Eo/B) differentiation in allergic subjects. In the present study, we investigated TLR-mediated changes in IL-25- (IL-17RB), IL-33- (ST2) and TSLP- (TSLPR) receptors by peripheral blood HPC in healthy and allergic asthmatic subjects. We also evaluated the effect of allergen challenge on TLR-induced responses.

Method: The study group comprised 10 healthy and 11 allergic asthmatic subjects. Asthmatics underwent diluent-controlled bronchial allergen challenge. PB was collected from both healthy and asthmatic subjects before and 24 hours after diluent/allergen challenge. PBHPC-enriched cell populations were stimulated with TLR-2 (lipoteichoic acid, LTA), TLR-4 (lipopolysaccharide, LPS) or TLR-9 (ODN2006) ligands. TLR-2, TLR-4 and TLR-9 expression by PBHPC as well as IL-17RB, ST2 and TSLPR expression after TLR ligation were examined by flow cytometry.

Results: Asthmatic HPC expressed significantly less TLR-2 and TLR-9, compared to non-asthmatic HPC ($p<0.05$), with a similar trend for TLR-4 ($p=0.057$). TLR-4 stimulation of asthmatic, compared to control, HPC yielded significantly higher expression of TSLPR ($p<0.05$), with a similar trend after TLR-9 stimulation ($p=0.054$). Conversely, stimulation of asthmatic HPC with ODN2006 led to a significant decrease in the percentage of IL-17RB expressing cells, compared with unstimulated cultures ($p<0.05$). Allergen challenge led to a further increase in TSLPR expression by LPS-stimulated PBHPC, compared to diluent ($p<0.05$) and ODN2006-stimulated PBHPC, compared with baseline ($p<0.05$). There was no effect of allergen inhalation on TLR-induced IL-17RB or ST2 expression.

Conclusions:

- The expression of TLR by PBHPC is decreased in asthmatics
- There is differential TLR-mediated expression of epithelial cytokine receptors by PBHPC of healthy vs. asthmatic subjects

TSLPR overexpression in response to TLR-stimulation after allergen challenge points to the involvement of HPC and TSLP in the initiation and persistence of airways inflammation during infection-driven asthma exacerbations. Similar studies with CBHPC will investigate whether these mechanisms are present in early life and thus serve to identify predictive biomarkers of atopic sensitisation and disease.

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Role of group 2 innate lymphoid cells and IL-13 in bronchial epithelial cell tight junction barrier leakiness

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Group 2 innate lymphoid cells (ILC2s) may play a role in asthma development independent of the adaptive immune system. Bronchial epithelial leakiness has been shown to be involved in asthma, however the role of ILC2 in the regulation of bronchial epithelial tight junctions (TJs) and barrier function was not known. Therefore, we sought to determine the role of ILC2s in bronchial epithelial TJ barrier. Co cultures of human ILC2s and air-liquid interface (ALI) cultures of primary bronchial epithelial cells were used to determine the measurement of transepithelial resistance (TER), paracellular flux, TJ mRNA and protein expressions and cytokines. To analyze the *in vivo* relevance of barrier disruption by ILC2s, the effect of ILC2s on TJs was examined using a murine model of IL-33-induced airway inflammation in wt, Rag-2$^{-/-}$, Rag-2$^{-/-}\gamma c^{-/-}$ and RORα-deficient Staggerer (Ror$\alpha^{sg/sg}$) mice, which is specifically deficient for ILC2s. ILC2s significantly reduced the TER and increased FITC-dextran permeability in ALIs after co-cultures, suggesting the induction of epithelial

leakiness. Consistently, ILC2s disrupted TJ proteins as well as decreased the expression of the mRNA of claudin-1, claudin-4, occludin and ZO-1. Neutralization of IL-13, but not IL-4 restored the impaired epithelial barrier function by ILC2s, suggesting that ILC2s induced human bronchial epithelial TJ barrier disruption through IL-13. The intranasal administration of recombinant IL-33 to wild-type and Rag-2$^{-/-}$ mice triggered TJ disruption in an ILC2- and IL-13-dependent manner as demonstrated by the analysis of cellular infiltration, broncho-alveolar lavage cell counts, lung mRNAs and confocal microscopy, whereas Rag-2$^{-/-}\gamma c^{-/-}$ and Ror$\alpha^{sg/sg}$ mice, which lack ILC2s did not recapitulate the response. These data demonstrate for the first time that ILC2s target bronchial epithelial TJ barrier as a novel mechanism in asthma pathogenesis.

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Allergenic proteases cleave the chemokine CX3CL1 directly from the surface of airway epithelium and augment the effect of Rhinovirus.

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Background: CX3CL1 (fractalkine) is a membrane-bound chemokine expressed mainly on endothelial and epithelial cells, and can be shed as a soluble chemotactic factor. In asthma, allergen challenge causes CD4$^+$ T cells to express CX3CR1, the CX3CL1 receptor, and CX3CL1 expression is increased in airway tissue suggesting that the CX3CL1/CX3CR1 axis may contribute to airway CD4$^+$ T lymphocyte recruitment. Since epidemiological evidence supports a synergistic interaction between virus infection and allergen exposure in precipitating asthma exacerbations, we postulated that rhinovirus (RV) exposure in the presence of allergen augments epithelial CX3CL1 release.

Methods: Fully differentiated primary bronchial epithelial cultures were grown at an air-liquid interface (ALI). They were pretreated with *Dermatophagoides pteronyssinus* house dust mite extract (HDM) or diluent and then infected with RV16 or UV-irradiated RV16. Recombinant HEK cells expressing full-length CX3CL1 were treated with HDM. Soluble CX3CL1 was measured by ELISA and western blotting. The mechanism of CX3CL1 shedding was assessed using protease inhibitors, fluticasone and PAR2 agonist peptide.

Results: Exposure to HDM and/or RV caused vectorial CX3CL1 release from ALI cultures. Basolateral CX3CL1 release was not affected by HDM but was significantly stimulated by RV16 replication; this was inhibited by fluticasone or GM6001 implicating involvement of NFκB and ADAM sheddases. In contrast, apical CX3CL1 shedding was stimulated by HDM alone and this was augmented by RV16. Although fluticasone or GM6001 modestly reduced the combined effect of RV16 and HDM on apical CX3CL1 release, heat inactivation or treatment of HDM with E64, a cysteine protease inhibitor, completely blocked CX3CL1 shedding. Since PAR2 agonist peptide did not stimulate CX3CL1 shedding, we postulated that HDM cysteine proteases directly cleaved CX3CL1 on the apical surface of the epithelium; this was confirmed by immunostaining and using CX3CL1-expressing HEK cells. Extracts of *Alternaria* fungus, but not grass pollen, also caused rapid shedding of CX3CL1.

Conclusions: We have identified a novel mechanism whereby allergenic proteases may directly affect CD4$^+$ T cell recruitment by cleaving CX3CL1 from the apical surface of the airway epithelium. As RV16 augmented HDM-induced CX3CL1 shedding, this may contribute to a synergistic interaction between allergen exposure and RV infection in triggering asthma exacerbations.

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Epithelial barrier defects and eosinophil extracellular trap formation in active eosinophilic esophagitis

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Background: Eosinophilic esophagitis (EoE) exhibits esophageal dysfunction owing to an eosinophil-predominant inflammation. Activated eosinophils generate eosinophil extracellular traps (EETs) able to kill bacteria. There is evidence of an impaired barrier function in EoE that might allow pathogens to invade the esophagus. This study aimed to investigate the presence and distribution of EETs in esophageal tissues from EoE patients and their association with possible epithelial barrier defects.

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

Results: EET formation occurred frequently and was detected in all EoE samples correlating with the numbers of infiltrating eosinophils. While the expression of both filaggrin and LEKTI was reduced, epithelial antimicrobial peptides (human beta-defensins-2, -3, cathelicidin

LL-37, psoriasin) and cytokines (TSLP, IL-25, IL-32, IL-33) were elevated in EoE as compared to normal esophageal tissues. There was a significant correlation between EET formation and TSLP expression (p=0.02) as well as psoriasin expression (p=0.016). On the other hand, a significant negative correlation was found between EET formation and LEKTI expression (p=0.016).

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

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Mechanisms of inducing and breaking allergen tolerance

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Although it is becoming more clear that the observed increment in the incidence of allergic diseases is due to the loss of peripheral T-cell tolerance to allergens, the mechanisms or specific cytokines or innate immune response stimulating factors involved in such processes remain largely unknown. We sought to identify molecular mechanisms that break allergen-specific T-cell tolerance in human subjects. In allergic patients the immune profile of the tonsils represents the atopic status of patients, with low expression of the TH1 cell-specific transcription factor T-bet and the cytokine IFN-g, as well as IL-10. Human tonsils show very low levels of allergen-induced T-cell proliferation, thus representing a very suitable in vivo model to assess mechanisms of breaking allergen-specific T-cell tolerance. Triggering of Toll-like receptor (TLR) 4 or TLR8 and the proinflammatory cytokines IL-1b or IL-6 break allergen-specific T-cell tolerance in human tonsils and peripheral blood through a mechanism dependent on the adaptor molecule myeloid differentiation primary response gene (88) (MyD88). In particular, myeloid DCs and stimulations that activate them broke the tolerance of allergen-specific CD4+ T cells, whereas plasmacytoid DCs and stimulations that activate them, such as TLR7 and TLR9, did not have any effect. Tolerance-breaking conditions induced by different molecular mechanisms were associated with a mixed cytokine profile with a tendency toward increased levels of IL-13 and IL-17, which are TH2 and TH17 cytokines, respectively.

Certain innate immune response signals and proinflammatory cytokines break allergen-specific CD4+ T-cell tolerance in normally unresponsive subjects, which might lead to the development or exacerbation of allergic diseases after encountering microbes or inflammatory conditions. The identification of the factors leading to the loss of peripheral allergen-specific T-cell tolerance and the better understanding of the pathways involved in such mechanisms will allow the design of alternative strategies aimed at preventing the development of allergic diseases, as well as improving the current treatment modalities.

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Characterization of Allergen, Chloroquine (CQ) and Histamine-Sensitive "Itch Nerves" Terminating in Mouse Skin.

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MrgprA3 is a G-protein coupled receptor (GPCR) stimulated by CQ that is expressed selectively in itch causing nerves in mouse skin (Cell, 2009,139:1353). Deleting only MrgprA3 expressing neurons inhibits not only CQ-induced scratching but also scratching caused by histamine and allergic reactions (Nat. Neurosci, 2013,16:174). We characterized the MrgprA3 nerves in a novel ex-vivo skin-dorsal root ganglia (DRG) preparation. Action potential discharges in single afferent nerves in the skin were evaluated by recording extracellularly from neurons within the DRG. CQ and histamine stimulated only C-fibers in the skin (CV=0.7 ± 0.02 m/s). Among 166 skin C-fibers studied (n=112 animals), 28% were CQ-sensitive itch nerves. CQ-sensitive fibers were histamine sensitive, and CQ-insensitive fibers were unresponsive to histamine.

Histamine, but not CQ, activated nerves in mrgpr-/- knock out mice. In sensitized mice, allergen (ovalbumin) activated the CQ-sensitive itch fibers, but did not stimulate CQ- insensitive, capsaicin-sensitive pain fibers. An RNAseq analysis of MrgprA3 expressing neurons revealed that among the 13 isoforms of phospholipase C (PLC), PLCβ3 predominates. CQ had little or no effect on skin C-fibers in plcβ3 -/- animals. Previous studies have revealed that TRPA1 is required for scratching in response to CQ (Nat. Neurosci. 2011, 14:595). However, we found no differences in the CQ-induced action

potential discharge in cutaneous itch C-fibers of wild-type vs. trpa1-/- animals (peak frequency discharges of 12 ± 2 Hz vs. 13 ± 2 Hz, respectively). Likewise no differences were noted in responses to CQ, histamine, or allergen in wild type vs. trpa1-/-/trpv1-/- double knock-out mice. The nonselective TRP channel blocker ruthenium red (30 μM) did not inhibit CQ-induced activation of skin C-fibers. These data indicate that allergen, histamine and CQ activate the same population of itch fibers in mouse skin. The GPCR- Gq dependent activation of itch fiber terminals requires PLCβ3 enzymes, but not TRPA1, TRVPV1 or other ruthenium red sensitive TRP channels. In studies where TRPA1 or TRPV1 inhibition blocks scratching in mice, it is likely that this is due to action at sites other than the itch nerve terminals in the skin (e.g. non-neural cells, or nerves in the spinal cord or brain).

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Enzymatically Active sADAM33 Is Increased In Asthma And Causes Airway Remodelling Without Inflammation

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Background: Asthma is a chronic inflammatory airway disease that involves genetic and environmental factors. *A Disintegrin and Metalloprotease-33 (ADAM33)* is a susceptibility gene associated with asthma, bronchial hyperresponsiveness (BHR) and reduced lung function. Although normally a membrane anchored protein, soluble forms of ADAM33 (sADAM33) containing the metalloprotease (MP) domain have been detected by immunoassay of bronchoalveolar lavage fluid (BALF). In asthma, BALF sADAM33 levels are increased and inversely correlate with FEV_1%. As little is known about the contribution of sADAM33 to asthma pathogenesis, we hypothesised that sADAM33 is enzymatically active in asthmatic airways and that sADAM33 promotes airway remodelling that leads to BHR and asthma.

Methods: We used Western blotting and a fluorescence resonance energy transfer (FRET) peptide cleavage assay to evaluate the presence of sADAM33 and its enzymatic activity in BALF from either patients with asthma or mice with house dust mite extract (HDM)-induced allergic airways inflammation. We assessed the functional role of ADAM33 using either a double transgenic (dTg) mouse model that conditionally (Dox-inducible) expresses human sADAM33 in lung epithelium or *Adam33* null mice. In the dTg model, sADAM33 expression was induced for 4 or 8 weeks, while the *Adam33* null mice were intra-tracheally challenged with HDM. BHR in response to methacholine was measured by FlexiVent. Pulmonary inflammation and remodelling were assessed by RT-qPCR and immunohistochemistry. BALF inflammatory cell counts were determined by differential counting; sADAM33 in BALF was assessed by Western-blotting and FRET peptide cleavage assay.

Results: Irrespective of corticosteroid treatment, enzymatically active sADAM33 was increased in BALF from asthmatic subjects, as well as mice with HDM-induced airway inflammation. Expression of the human sADAM33 transgene in adult mouse lungs caused airway remodelling without any evidence of airway inflammation. Conversely, in *Adam33* null mice challenged with HDM there was suppression of airway remodelling. Furthermore, eosinophilic airway inflammation and BHR were reduced.

Conclusion: Our data suggest that enzymatically active sADAM33 plays an important role in the pathogenesis of asthma by causing airway remodelling which is independent of inflammation. Since loss of ADAM33 suppresses both airway remodelling and inflammation, sADAM33 is a novel target for disease modifying therapy in asthma.

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sADAM33 Causes Airway (P)remodeling In Early Life And Enhances Eosinophilic Airway Inflammation And Bronchial Hyperresponsiveness

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Background: Most asthma has its origins in early life. Asthma is a complex disease involving gene-environment interactions. *ADAM33* is a susceptibility gene associated with asthma, bronchial hyperresponsiveness (BHR) and reduced lung function. The gene encodes a membrane-anchored metalloprotease (MP) whose ectodomain can be shed as a soluble protein (sADAM33), which is increased in asthma. While sADAM33 is induced *in utero* by maternal allergy, little is known about how it affects the structure or function of developing airways. We hypothesised that sADAM33 promotes airway remodeling in early life, making the lungs more susceptible to development of allergic inflammation and asthma.

Methods: We used double transgenic (dTg) mice that conditionally (Doxycycline-inducible) express human sADAM33 in lung epithelium. sADAM33 was induced from *in utero* until 8 weeks *post partum,* then the dTg mice (or single transgenic, sTg, controls) were challenged with recombinant IL-13 or sub-threshold doses of house dust mite extract (HDM). Airway resistance in response

to methacholine was measured by FlexiVent. Inflammatory cell counts and mediators in bronchoalveolar lavage fluid (BALF) were quantified. Lung tissue was analysed by RT-qPCR and immunohistochemistry (IHC). Human embryonic lung explants were cultured in the presence of highly purified recombinant, enzymatically active sADAM33 or inactive sADAM33 (E346A) for 12 days before IHC analysis.

Results: When induced from *in utero*, sADAM33 caused increase in bronchial smooth muscle and airway remodelling (termed (p) remodeling) in the absence of inflammation or airway resistance. However, when dTg mice were challenged with IL-13, lung eotaxin levels were higher than sTg mice. Furthermore, dTg mice with (p)remodeled airways responded to low dose HDM challenges with significant increases in eosinophilic airway inflammation, Th2 inflammatory mediators, remodelling genes and methacholine-induced airway resistance compared to control. Exposure of human embryonic lung to recombinant sADAM33 caused increase in bronchial smooth muscle compared to explants treated with inactive sADAM33 (E346A).

Conclusion: Our data challenge the conventional paradigm that airway remodelling is a consequence of inflammation. We propose a new paradigm in which ADAM33 (p)remodeled airways provide the 'soil' for allergic inflammation in susceptible individuals leading to Th2–type allergic airway inflammation and BHR that characterizes asthma early in life.

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The allergic pre-commitment of the airways

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The airway epithelium is the first line of defense and is continuously exposed to environmental factors. It is currently unknown if in allergy-conditioned epithelium equally responses to environment compared to non-allergic triggers.

We therefore investigated airway epithelial cells under the influence of the Th2-cytokine IL-4 in comparison to the Th1-cytokine IFN-γ. The antagonistic crosstalk between IL-4 and IFN-γ is one of the key paradigms that are used to explain allergic sensitization on the level of lymphocytes. Our studies show for the first time that not only lymphocytes but also primary human airway epithelial cells are subjected to an antagonistic cross-regulatory network that induces a Th2- and Th1-specific response profile. Genome-wide profiling revealed that IL-4 induced factors are stringently downregulated by IFN-γ, and vice versa. This network is based on the well-known Th2 transcription factor *GATA-3*, but also yet unknown Th2-induced transcriptional regulators *MEIS-1* and *HEY-2* that appear as hub genes in a network of Th2 driven network.

In contrast, the Th1 transcription factor *T-BET* is less involved whereas *TEAD-4* appears as a novel transcriptional hub-regulator in Th1-driven network.

Interestingly, the presence of both cytokines did not induce a substantial number of synergistically regulated genes. In contrast, the co-presence revealed a large number of antagonistically regulated genes, while several genes escaped the antagonistic repression, such as *IL-24*, *CSF-3*, *CXCL-6*, *CHI3L1* (YKL40) and *CCL-17*. These genes have a particular biomarker potential as they are secreted and not influenced by the antagonism. In fact, nasal secretions as well as induced sputum supernatants show increased IL24 levels in season as opposed to samples taken out of season underlining the biomarker potential of epithelium-derived gene products. Taken together, the current study demonstrates that airway epithelial cells are committing to a Th2-induced phenotype that is antagonized by IFN-γ.

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Predisposition to asthma due to environmental priming of natural killer cells in utero

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Background: Maternal environmental exposures, including exposure to diesel exhaust are increasingly recognized as important risk factors for childhood asthma. The mechanisms are poorly characterized. We sought to address this gap in knowledge.

Methods: To build a framework for mechanistic studies we have developed two mouse models. In the "pregnancy" ("P") model, C57BL/6 mice received an intranasal application of diesel exhaust particles (DEP) in phosphate-buffered saline (PBS) on days 3, 6, 9, 12, 15 and 18 of pregnancy. In the "pre-pregnancy" ("pre-P") model, the repeated maternal exposures occurred before pregnancy. Two weeks after the final exposure, females were mated with unexposed males. There were no subsequent exposures during pregnancy. In both models, offspring underwent immunization and challenge with ovalbumin (OVA). The immunization was "suboptimal" as it occurred during the neonatal period. Control mothers and pups received PBS. Pups were examined for airway hyperresponsiveness to methacholine (FlexiVent), peribronchial cellular infiltrates (histology), cell counts in the bronchoalveolar lavage fluid (BALF) and expression of intracellular IL-5, IL-13 and IL-17 in three pulmonary leukocyte (CD45+) populations: CD4 T cells, natural killer (NK) T and NK cells.

Results: Maternal exposure to DEP either before or during pregnancy predisposed offspring to asthma. Compared to OVA-treated pups of PBS-exposed mothers (PBS-OVA group), the OVA-treated pups of DEP-exposed mothers (DEP-OVA group) had larger

peribronchial infiltrates (3.4 and 1.8 fold increase in "P" and "pre-P" models, respectively) and increased counts of eosinophils in the BALF (5.5 and 3.2 fold increase in "P" and "pre-P" models, respectively). Furthermore, compared to PBS-OVA pups, DEP-OVA pups manifested significantly higher airway resistance in response to methacholine. In DEP-OVA mice from the "P" model the majority (>50%) of cytokine (IL-5, IL-13, IL-17)-positive pulmonary leukocytes were NK cells. Antibody-mediated depletion of NK cells prevented development of airway inflammation and hyperresponsiveness in both models.

Conclusions: We have developed two mouse models linking pregnancy and pre-pregnancy exposure to DEP with predisposition to asthma in offspring. This predisposition to asthma is driven by type 2 cytokine- and IL-17-producing NK cells.

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Interleukin-36γ: a novel target for severe asthma?

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Background: Conventional asthma treatment involves the usage of inhaled corticosteroid and bronchodilator to prevent swelling and relax airway muscles. However approximately 20% of these patients non-responsive to treatment and have increased risk of hospitalization and death. Recent advances in understanding of asthma have discovered critical cytokines that linked to innate immune activation and development of steroid resistant asthma. Our studies identified novel roles of interleukin (IL)-36γ in regulating airway inflammation. We hypothesize that the pro-inflammatory effects of the innate immune cytokine, IL-36, may be important in pathogenesis of severe asthma.

Methods: Naïve mice were administered with recombinant IL-36 intranasally to characterize its functional role in lungs. In a separate model, mice were sensitised and challenged with ovalbumin (OVA) to induce hallmark features of asthma. During allergen challenge, recombinant IL-36γ was administered. As endpoints, airway hyperresponsiveness will be measured using Buxco FinePointe series resistance and compliance. Airway inflammation will be assessed by histological changes, BALF and lung cells infiltration, cytokines and chemokines production.

Results: Interestingly, administration of recombinant IL-36γ, *in vitro* (bronchial epithelial cells) and *in vivo* (lungs), increased TNF-α, IL-1β, IL-6, IL-33, IL-13, and CXCL1 expression. Treatment with IL-36γ also increased recruitment of type 2 innate lymphoid cells (ILC2), eosinophils, and dendritic cells (DC), which are critical for asthma pathogenesis. We next sought to determine the importance of IL-36γ in asthma using a well-characterised murine allergic airway disease model. Intriguingly, mice treated with IL-36γ became insensitive to corticosteroid therapy. To determine the clinical relevant of our study, we have looked at the expression of IL-36γ in sputum of asthmatics. IL-36γ expressions were found to be higher in asthmatics with neutrophilic inflammation (found predominantly in severe asthmatics).

Conclusion: Infection can augment inflammation in lungs of asthmatics by increasing inflammatory cytokines production, or inflammatory cell recruitment, which worsen the symptoms of asthma. In our model we can show that infections that trigger asthma exacerbations are linked to IL-36 production and that independently delivery of this cytokine to the airways results in steroid resistant AHR and neutrophilic inflammation. Our results suggest that IL-36γ could be a potential therapeutics target for severe asthmatics.

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Acute lipopolysaccharide exposure during a severe paramyxoviral respiratory infection is sufficient to prevent development of post-viral atopic disease.

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The cause(s) of increasing atopic diseases in the westernized world is unknown; the hygiene and viral hypotheses have each been put forward to explain the increase. Using a mouse model of paramyxoviral induced atopy, we tested the hypothesis that exposure to bacterial products during a severe respiratory viral infection would be sufficient to prevent the development of post-viral atopic disease – essentially marrying the hygiene and viral hypotheses. Previously, we demonstrated infection with the paramyxovirus, Sendai virus (SeV), led to recruitment of CD49d expressing neutrophils (PMN), which were necessary for subsequent development of post-viral atopic disease. In contrast, lung lipopolysaccharide (LPS) exposure recruited PMN lacking CD49d, and did not lead to atopic disease. We assessed whether LPS exposure during SeV infection would reduce accumulation of CD49d$^+$ PMN. LPS (3 μg) given i.n. at 60 hours post SeV reduced frequency of CD49d$^+$ PMN by ~80% compared to PBS ($p<0.05$; n=3). We assessed whether this reduction in CD49d$^+$ PMN attenuated post-viral atopic disease. C57BL6 mice were inoculated with SeV ($2x10^5$ pfu) or UV-inactivated SeV (UV-SeV); 60 hours post inoculation (PI) 3 μg LPS or PBS was administered i.n. Twenty-one days PI airway hyper-reactivity and mucous cell metaplasia were determined. LPS exposure significantly reduced airway hyper-responsiveness (SeV+PBS versus SeV+LPS, $p=0.02$; SeV+PBS versus

UV-SeV+PBS, p=0.001; SeV+LPS versus UV-SeV+LPS, p=0.83; n≥4 mice per group) and mucous cell metaplasia (mean±SEM PAS$^+$ cells/mm BM: SeV+PBS 5.2±0.6; SeV+LPS 2.0±0.5, p=0.003 versus SeV+PBS; UV-SeV+PBS 0.5±0.2, p<0.0001 versus SeV+PBS; UV-SeV+LPS 0.6±0.4, p=0.09 versus SeV+LPS; n≥4 mice per group). Therefore, acute exposure to a bacterial product (LPS) early in a respiratory viral infection is sufficient to prevent development of post-viral atopic disease. Further studies will examine whether there is a difference between chronic and acute LPS exposure in this model system.

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The potential for repositioning anti-thyroid agents as anti-asthma drugs

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Background: Various anti-asthma drugs targeting type 2 immune mediators—IL-4, IL-5, IL-13, and CRTH2—are now under development. However, to develop novel drugs, particularly biologics, requires huge investments of time and money and involves safety risks. Drug repositioning, the process of finding new therapeutic indications for existing drugs, is highly desirable as an alternative strategy. However, to our knowledge, there has been thus far no instance of drug repositioning in bronchial asthma. Airway peroxidase functions as a potent defense system against microbes by producing biocidal compounds. In contrast, the deleterious effects of the airway peroxidase system in bronchial asthma remain undetermined.

Methods: We first examined expression of three airway peroxidase—myeloperoxidase (MPO), eosinophil peroxidase (EPX), and lactoperoxidase (LPO) expressed at neutrophils, eosinophils, and epithelial cells, respectively—in a mouse model of airway allergic inflammation and in the bronchial biopsy samples from ten asthma patients. We then applied 2-mercapto-1-methylimidazole (methimazole), an agent that inhibits all peroxidases and is widely used as an anti-thyroid agent targeting thyroid peroxidase, to the model mice. We adopted two protocols, a long administration (orally every day from the start of sensitization) and a short administration (orally every day from two days before the start of allergen airway challenge).

Results: Pulmonary expression of all three peroxidases—*Mpo*, *Epx*, and *Lpo*—were significantly up-regulated in the allergen-challenged mice. Although the expression levels of *LPO* varied, some patients showed distinctly high levels of expression, suggesting heterogeneity of airway peroxidase expression among asthma patients. Parameters of OVA-induced airway inflammation (i.e., AHR, infiltration of inflammatory cells in BALF, and histological changes) were completely inhibited in the long administration of methimazole and less so (yet significantly) in the short administration in the model mice.

Conclusions: These results suggest that peroxidase activities are critical for the setting of airway allergic inflammation in the model mice and that anti-thyroid agents can be repositioned as anti-asthma drugs.

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Glucagon like peptide-1 receptor signaling inhibits both lung innate and adaptive immunity mediated allergic inflammation to inhaled aeroallergen

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Rationale: Cells of both the innate (ILC2) and adaptive (T lymphocytes) immune responses robustly produce the Th2 cytokines IL-5 and IL-13, cytokines central to the asthma phenotype. However, the effect of glucagon-like peptide 1 (GLP-1) receptor signaling on Th2 cytokine secretion in response to an inhaled protease containing aeroallergen is unknown.

Objectives: To determine the effect of GLP-1 receptor signaling on mouse lung innate and adaptive Th2 inflammation in response to inhaled aeroallergen.

Results: In a mouse model of innate immunity-driven allergic airway inflammation induced by 4 consecutive days of airway challenge with an extract of *Alternaria alternata*, a fungal aeroallergen associated with severe asthma exacerbations, a subcutaneously administered GLP-1 receptor agonist significantly inhibited airway IL-33 protein expression, blunted lung IL-5 and IL-13 protein expression, reduced the number of lung IL-5 and IL-13 expressing ILC2, and decreased the number of airway eosinophils. In addition, in a model of adaptive immunity-driven allergic response, GLP-1 receptor signaling significantly inhibited serum IgE, reduced the number of lung IL-5 and IL-13 expressing CD4+ T lymphocytes, decreased airway eosinophils and mucus expression.

Conclusions: These results suggest that GLP-1 receptor agonists are a therapeutic target to reduce both the innate and adaptive allergic responses to protease-containing aeroallergens such as *Alternaria*. GLP-1 receptor agonists are approved for the treatment of obesity and non-insulin dependent diabetes and may be particularly useful for asthma treatment in obese individuals.

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Early introduction of egg with two step procedure for infants with atopic dermatitis to prevent egg allergy: A double-blind placebo-controlled randomized clinical trial

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Background: Early introduction of solid foods for high risk infants with atopic dermatitis was revealed to have protective effect from onset of food allergy at later life. However, prevention from very early onset of infantile food allergy who already sensitized at the introduction point still needs to be explored.

We investigated whether early introduction of a small amount of egg with two steps procedure for infants with atopic dermatitis would prevent egg allergy safely in the first year of life.

Methods: This trial was designed as a double-blind, placebo-controlled, parallel-group, randomized clinical trial. Four or five month old infants with atopic dermatitis were recruited and randomly assigned to one of the two groups: placebo or egg consumption group. Participants of egg group took 50 mg of heated egg powder, equivalent to 0.2g of boiled egg, from 6 to 9 months and 250 mg thereafter from 9 to 12 months of age. The primary outcome was prevalence of hen's egg allergy confirmed by an oral food challenge at 12 months old. Eczema of the participants in both groups was thoroughly treated.

Results: This trial was terminated based on the result of scheduled interim analysis which showed significant difference between the two groups. Intention-to-treat analysis for the 121 participants revealed that the prevalence of egg allergy was 37.7% in placebo group (n=61) and 8.3% in egg group (n=60) (p=0.0013). No one experienced adverse reaction to intervention powder in both groups. Most of the participants' skin conditions were well maintained to achieve flare prevention.

Conclusions: The introduction of a small amount of heated egg at six months old followed by two steps intake was effective and safe for infants with atopic dermatitis to prevent hen's egg allergy in the first year of life. The trial registration is UMIN-CTR 000008673.

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Heterogeneity of Airway Type 2 Innate Lymphoid Cells (ILC2s) and Their Steroid Responsiveness in Asthma

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Background: ILC2s are an important source of type 2 cytokines. Mouse ILC2s are heterogeneous. Heterogeneity of human ILC2s and their contribution to asthma heterogeneity and steroid resistance is unknown.

Methods: We used bronchoalveolar lavage and blood cells, and not-fit-for-transplantation donor lungs to characterize human ILC2 subpopulations by flow cytometry.

Results: Human ILC2s are identified as Lin-CRTH2+ cells. We identified a novel subpopulation of ILC2s in the human lung, BAL and blood that did not express CRTH2. These Lin-CRTH2- cells expressed known ILC2 markers—IL7Rα, IL25R, GATA3, Bcl11b, PLZF, KLRG1 and c-kit. They produced IL5 and IL13 but, unlike CRTH2+ cells, produced very little IL4 and amphiregulin. The frequency of IL5+ CRTH2+ and IL5+ CRTH2- ILC2s were 0.21±0.07% and 0.12±0.03%, respectively, in BAL from asthmatic patients (N=18), and 0.10±0.04% and 0.11± 0.03%, respectively, from disease controls (N=14). The difference in IL5+CRTH2+ ILC2s between asthma and disease controls was significant (P=0.01). IL5+ILC2s correlated (r=0.62, P=0.04) with BAL eosinophils. Adoptive transfer of human lung CRTH2- ILC2s to Rag2-/-:gc-/- mice followed by Alternaria allergen challenge produced airway hyperreactivity (4-fold increase in R_L) that was similar to that with the transfer of CRTH2+ ILC2s.

ILC2s manifested a heterogeneous steroid response. Dexamethasone (dex) inhibited blood IL5+ ILC2s by 77±12% but BAL IL5+ILC2s by only 21±14% (N=16) suggesting a relative steroid resistance. The ILC2 subpopulations had a similar steroid response. IL7 and TSLP (both are IL7Rα ligands) but not IL2, IL25 or IL33 induced steroid resistance in IL5+ILC2s in vitro. Dex increased IL7Rα expression by 171±16% in ILC2s and reduced the threshold for IL7- and TSLP-induced STAT5 activation by half. STAT5 interacts with the glucocorticoid receptor and blocks its nuclear function. We observed sustained (lasting >7 days) STAT5 phosphorylation in IL7/TSLP but not IL2/IL33-stimulated ILC2s. Tofacitinib, a clinically available Jak-STAT inhibitor, reversed steroid resistances of ILC2s. TSLP was elevated in BAL from select asthmatic patients, which negatively correlated (r=-0.62) with dex-inhibition of BAL IL5+ILC2.

Conclusions: We identified a novel subpopulation of ILC2 that produce select type 2 cytokines and induce asthma. IL7 & TSLP induce steroid resistance in ILC2s in a STAT5-dependent manner. Steroid resistant ILC2s are associated with TSLP-high asthma.

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Allergen endotoxins induce T cell-dependent and non-IgE-mediated nasal hypersensitivity in mice

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Background: Allergen-mediated cross-linking of IgE on mast cells/basophils is a well-recognized trigger for type-1 allergic diseases such as allergic rhinitis (AR). However, allergens may not be the only trigger for AR, and several allergic-like reactions are induced by non-IgE-mediated mechanisms. Here, we describe a novel non-IgE-mediated, endotoxin-triggered nasal type-1-hypersensitivity-like reaction in mice.

Methods: To investigate whether endotoxin affects sneezing responses, mice were intraperitoneally immunized with ovalbumin (OVA), and then nasally challenged with endotoxin-free or endotoxin-containing OVA. To investigate the role of T cells and mechanisms of the endotoxin-induced response, mice were adoptively transferred with *in vitro* differentiated OVA-specific Th2 cells, and then nasally challenged with endotoxin-free or endotoxin-containing OVA.

Results: Endotoxin-containing, but not endotoxin-free, OVA elicited sneezing responses in mice independent from IgE-mediated signaling. OVA-specific Th2 cell adoptive transfer to mice demonstrated that local activation of antigen-specific Th2 cells was required for the response. The Toll-like receptor 4-myeloid differentiation factor 88 -signaling pathway was indispensable for endotoxin-containing OVA-elicited rhinitis. In addition, lipopolysaccharide directly triggered sneezing responses in OVA-specific Th2-transferred and nasally endotoxin-free OVA-primed mice. Although an antihistamine, diphenhydramine, suppressed sneezing responses, mast-cell/basophil-depleted mice had normal sneezing responses to endotoxin-containing OVA. Clodronate treatment abrogated endotoxin-containing OVA-elicited rhinitis, suggesting the involvement of monocytes/macrophages in this response.

Conclusions: Antigen-specific nasal activation of $CD4^+$ T cells followed by endotoxin exposure induces mast cell/basophil-independent histamine release in the nose that elicits sneezing responses. Thus, environmental or nasal residential bacteria may exacerbate AR symptoms. In addition, this novel phenomenon might explain currently unknown mechanisms in allergic (-like) disorders.

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The interplay of Bet v 1 and birch pollen lipids in the allergic sensitization process

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Background: The major birch pollen allergen Bet v 1 possesses a large hydrophobic cavity which is thought to be responsible for transport or storage of fatty acids, flavonoids or cytokinins. Lipids co-delivered with allergens can have a significant impact on the allergic sensitization process. We aimed to analyze the ability of Bet v 1 to bind lipids from different sources and to investigate the phosphorylation profiles of kinases in primary keratinocytes after stimulation with Bet v 1 alone or in combination with lipids.

Method: Total lipids from peanut, birch and grass pollen were extracted using chloroform/methanol. The concentration was determined by using the sulfo-phospho-vanillin method. rBet v 1.0101 (10 µM final concentration; 174 µg/mL) was incubated with 3 µg/mL, 15 µg/mL and 30 µg/mL of lipid extracts. Lipid binding to Bet v 1 was monitored by adding 10 µM 1-anilinonaphthalene-8-sulfonic acid (ANS) and measuring the decrease of fluorescence at 484 nm. Primary keratinocytes were obtained from abdominal skin. The phosphorylation profiles of 43 kinases were analyzed by using the Human Phospho-Kinase Antibody Array.

Results: A concentration dependent reduction of ANS binding was observed after incubating Bet v 1 with lipid extracts from birch or grass pollen (76% or 73% with 30 µg/mL lipids, respectively). This was exclusively observed for pollen but not for peanut lipids. Furthermore, we showed that the phosphorylation levels of p38α, GSK-3α/β, Akt, ERK1/2, p70 S6 Kinase, and CREB were increased after stimulation with Bet v 1 in combination with birch pollen lipids compared to the allergen or the lipids alone. The increased phosphorylation of CREB after treatment with Bet v 1 and birch pollen lipids was moreover confirmed by Western Blot.

Conclusion: We observed that lipids from birch and grass pollen were able to bind to the hydrophobic cavity of Bet v 1. Furthermore, we showed that in primary keratinocytes a panel of kinases revealed increased phosphorylation levels after treatment with Bet v 1 in combination with birch pollen lipids. This might represent an important mechanism by which pollen lipids may act as potential danger signals during the sensitization phase.

Supported by Austrian Science Fund SFB-F4608.

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Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence – a BAMSE/MeDALL study

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Background: Sensitization to individual cat and dog allergen molecules may contribute differently to development of allergy to these animals. The aim of the study was to investigate the association between sensitization patterns to cat and dog allergen molecules during childhood and symptoms to these furry animals up to 16 yrs.

Methods: Data from 779 randomly collected children from the BAMSE birth cohort at 4, 8 and 16 years were used. IgE to cat and dog using ImmunoCAP and allergen molecules using allergen chip based on ISAC technology (MeDALL chip). Allergy was defined as reported rhinitis, conjunctivitis or asthma at exposure to cat or dog.

Results: Cross-sectionally, IgE to Fel d 1 and to cat extract had similar positive predictive values (PPV) for cat allergy. IgE to Can f 1 showed a higher PPV for dog allergy than dog extract IgE. Sensitization to Fel d 1 and to Can f 1 in childhood were significantly associated with symptoms to cat or dog at 16 yr. Polysensitization to three or more allergen molecules from cat or dog (respectively) was a better longitudinal predictor of cat or dog symptoms than IgE testing with cat or dog allergen extract (respectively). Cross sectionally, cat-dog polysensitized children had higher IgE-levels and more frequent symptoms to cat and dog than monosensitized.

Conclusions: Sensitization to Fel d 1 and Can f 1 in childhood as well as polysensitization to either cat or dog allergen molecules predict cat and dog allergy cross-sectionally and longitudinally significantly better than IgE to cat or dog dander extract.

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Component-based analysis of IgG4 antibodies suggests that the low capacity of the Immuno Solid-Phase Allergen Chip microarray limits detection of specific IgE antibodies in patients with eosinophilic esophagitis

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Background: The ImmunoCAP nitrocellulose immunosorbent accommodates ~5,000 times more antigen than is immobilized on the Immuno Solid-Phase Allergen Chip (ISAC) microarray. Recently, two groups reported a low prevalence of food sensitization among adults with eosinophilic esophagitis (EoE) using ISAC. In a completely separate investigation, elevated serum IgG4 antibodies to whole food allergens, including cow's milk, were observed in adults with EoE. Presented is component-based analysis focusing on IgE, pan-IgG, and IgG4 antibodies to cow's milk components Bos d 4 (alpha-lactalbumin), Bos d 5 (beta-lactoglobulin), Bos d 6 (bovine serum albumin/BSA), and Bos d 8 (caseins) in sera from subjects with EoE.

Methods: IgE and IgG4 antibodies to cow's milk components were measured by ImmunoCAP. Specific Pan-IgG and IgG4 antibodies to alpha-lactalbumin or beta-lactoglobulin were measured from sera by selective adsorption onto Sepharose conjugated to recombinant Protein G or mouse anti-human IgG4, respectively, followed by incubation with radiolabelled antigen. Qualitative serological differences were assessed by comparing IgG4 antibodies and IgG4:IgE ratios to relevant allergens (i.e., galactose-alpha-1,3-galactose, Ara h 2, Fel d 1) in subjects with either food anaphylaxis or indoor cats.

Results: IgG4 and pan-IgG antibodies to alpha-lactalbumin and beta-lactoglobulin were strongly correlated in subjects with EoE (r_s>0.95, p<0.001). The results of the anti-human IgG4-Sepharose assays for IgG4 antibodies to alpha-lactalbumin or beta-lactoglobulin confirmed those obtained by ImmunoCAP (r_s>0.90, p<0.001). ImmunoCAP revealed that IgG4 antibodies to cow's milk components were higher in titer and IgG4:IgE ratios were ≥100-fold greater compared with IgG4 and ratios to relevant allergens in subjects with food anaphylaxis. Among subjects with EoE, the geometric mean of IgG4 antibodies to alpha-lactalbumin, beta-lactoglobulin, and caseins by ImmunoCAP

were >20 mg_A/l, which is ~10,000 times the mass of antigen used on an ISAC plate. Serum IgG4 antibodies to alpha-lactalbumin and beta-lactoglobulin comprised >60% of specific pan-IgG antibodies in subjects with EoE. The IgG response to alpha-lactalbumin and beta-lactoglobulin in EoE was only similar to that to Fel d 1 in individuals with indoor cats.

Conclusions: Antigen-specific IgG antibodies should be considered when selecting appropriate assays for serum IgE antibodies, particularly in situations where IgE is very low relative to IgG4 of the same specificity.

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Relation of serum and sputum IL-33 levels with sputum inflammatory cells and lung function in bronchial asthma

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Background: Th2-deviated airway inflammation is a characteristic of asthma, and IL-33 is one of the important cytokines that cause innate Th2 immune deviation. IL-33 induces production of IL-5 and IL-13 leading to recruitment of eosinophils into the airway and promotion of airway remodeling in asthmatics. However, few studies have evaluated the relation of serum and sputum IL-33 level with clinical features of asthma. Therefore, we aimed to compare the serum IL-33 level in the asthma patients and normal controls, and to find the correlation of the levels in sputum with physiologic changes and airway inflammatory patterns in asthma patients.

Methods: Asthmatics defined by GINA guideline and normal controls having no respiratory medical history were enrolled. Serum was obtained from both groups, and hypertonic saline-induced sputum was obtained only from asthma patients. Differential cell count of sputum was done. IL-33 was measured using sandwich ELISA. The levels were analyzed with respect to airway flow limitation and inflammation patterns (macrophage, neutrophil and eosinophil % in sputum).

Results: sputum IL-33 showed negative correlations with FVC and FEV1 (n=118, ρ=-0.193, p=0.036 and ρ=-0.184, p=0.045, respectively) in asthmatics. The levels also showed a positive correlation with sputum eosinophil (ρ=0.198, p=0.033), but not with macrophages or neutrophils. In serum, IL-33 level was significantly increased in asthmatics compared to normal controls (5.14 ±6.79 vs 1.83 ±2.91 ng/mL, p=0.013). Serum IL-33 also showed a negative correlation with FEV1 (n=103, ρ=-0.215, p=0.029), but neither with FVC nor peripheral blood eosinophil counts in asthma patients.

Conclusions: IL-33 in airways and peripheral bloods may be related with the airflow limitation of the asthmatics airway, and IL-33 in the airways may be one of the determinants for eosinophilic airway inflammation.

Key Words: Asthma;Interleukin-33, human;Lung function test; Induced sputum

Acknowledgement: The biospecimens and data used for this study were provided by the Biobank of Soonchunhyang University Bucheon Hospital, a member of the Korea Biobank Network.

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CD48 and asthma: association and potential biomarker

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Background: We have previously found that mast cells and eosinophils express CD48, a CD2 family GPI-anchored cell surface receptor (mCD48). Moreover, the binding of mCD48 by the high affinity ligand 2B4 (expressed on eosinophils) and by *S. aureus*/toxins induces their activation. In a murine model of allergic asthma, CD48 is a signature gene, neutralization of which significantly decreases allergen-induced lung inflammation.

Methods: In this study we evaluated the expression and possible role of CD48 in asthmatic patients with different disease severity. mCD48 on peripheral blood leukocytes (FC) and soluble CD48 (sCD48) in serum (ELISA) were evaluated in a group of mild/moderate asthmatics (Israeli cohort, IL). sCD48 was also measured in a UK cohort of mild, moderate and severe asthma and in healthy controls (Wessex Severe asthma cohort study). Bronchial biopsies from asthmatics were stained for CD48 (ICH). *In vitro,* eosinophil sCD48 production and its effects on 2B4-dependent eosinophil activation (EPO and IL-8 release) were evaluated.

Results: Expression of mCD48 was significantly elevated on eosinophils in mild and moderate asthma and on neutrophils in moderate asthma. Significantly increased sCD48 levels were found in mild asthmatics (IL and UK) compared to healthy controls. Significantly reduced sCD48 levels were detected in moderate (low–intermediate dose ICS treated) and in severe (high ICS + oral CS) asthma when compared to the mild disease. sCD48 did not correlate with atopy, peripheral blood eosinophil counts, IgE, gender or smoking behavior. An optimal cut-off value for sCD48 in both the IL and UK population was identified (>1482 pg/ml and >1619 pg/ml, 87.67% and 85.71% sensitivity, 60.87% and 60.0 % specificity, positive likelihood ratio of 2.21 and 2.14, respectively) for differentiating asthma from health. In bronchial biopsies from asthmatics, mCD48 was expressed predominantly by eosinophils. *In vitro* activated eosinophils released sCD48 and pre-incubation with sCD48 inhibited anti-2B4 induced eosinophil peroxidase and IL-8 release, and phosphorylation of Vav-1.

Conclusions: mCD48 and sCD48 were elevated in patients with mild asthma and sCD48 was decreased in moderate/severe asthma treated with corticosteroids. These findings suggest that CD48 is relevant in human asthma and may be useful as a biomarker of the disease.

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Potential Biomarkers Representing Subtypes of Aspirin Exacerbated Respiratory Disease(AERD)

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AERD refers to the development of bronchoconstriction and rhinitis with/without urticaria if exposed to ASA/NSIADs in patients with chronic asthma. Key features of AERD are overproduction of cys-LTs and intense eosinophilic infiltrations in upper and lower airways, which are associated with higher prevalence of severe asthma and chronic rhinosinusitis(CRS).

A two-step cluster analysis analyzing 302 AERD patients enrolled at Ajou University Hospital cohort demonstrated 4 different subtypes, in which atopic status, female ratio, and the prevalences of CRS/nasal polyp, chronic urticaria and severe asthma were significantly different among 4 subtypes. Moreover, anti-asthmatic medication requirements including high dose ICS/LABA and systemic steroid, and peripheral /sputum eosinophil counts were significantly different among 4 subtypes.

Metabolomic analysis demonstrated that urinary LT levels were significantly higher in subtype 1&3, while 15-LO metabolites were significantly higher in subtype 3. Mast cell-derived metabolite was significantly higher in subtype 2. Genomic analysis showed significant differences in the genotype of HLA-DPB1 12080 G>A among 4 subtypes. Urinary LTE4 metabolite levels were significantly higher in AERD patients carrying HLA DPB1*0301 and CysLTR1-634 T allele, both of which were strong gene markers for AERD.

These findings suggest that stratified medicine with applying biomarkers of each subtype will be able to achieve better outcome in the management of AERD, leading to precision medicine.

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MicroRNA-146a and microRNA-155 expression in induced sputum and blood of allergic asthmatics

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Background: MicroRNAs (miRs) are small noncoding RNAs that regulate gene expression and are emerging as modulators of immune responses. We have previously demonstrated that miR-155 deficiency results in decreased eosinophilic inflammation, T_H2 cells and T_H2 cytokine production locally in the lung in a mouse model of allergic airway inflammation. Furthermore, miR-155 has been suggested to enhance pro-inflammatory responses while miR-146a is suggested to act anti-inflammatory. The aim of this study was to address whether miR-146a or miR-155 is differently expressed in induced sputum or blood of allergic asthmatics compared to healthy control individuals.

Methods: Induced sputum and blood samples were obtained from stable allergic asthmatics and healthy control volunteers from the cohort West Sweden Asthma Study. Peripheral blood mononuclear cells (PBMCs) were isolated and *in vitro* stimulation with anti-CD3/CD28 or saline was performed. MiR-146a and miR-155 expression in sputum supernatants, PBMC supernatants and in PBMCs were determined by qPCR.

Results: MiR-155 expression was significantly down regulated in sputum supernatants from stable allergic asthmatics compared to healthy control individuals ($p<0.05$). In contrast, *in vitro* stimulated PBMC supernatants from both allergic asthmatics and healthy controls demonstrated a significant upregulation of miR-155 and a downregulation of miR-146a expression, respectively ($p<0.05$), and similar results were obtained in the *in vitro* stimulated PBMCs.

Conclusions: Our data demonstrates differential expression of miR-155 in the airways of allergic asthmatics compared to healthy control individuals, which may indicate a regulatory role in the airways of allergic asthmatics. However, further studies are needed to understand the contribution of miRs to the pathogenesis of human allergic airway disease such as allergic asthma.

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Distinct plasma chemokine levels in non-IgE-mediated gastrointestinal food allergy, compared with IgE-mediated food allergy

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Rationale: The number of reports on non-IgE-mediated gastrointestinal food allergy (non-IgE GI FA) cases has increased in recent decades. We, previously, reported that the antigen-specific stimulation on PBMCs from non-IgE GI FA patients lead to Type 2-predominant immune response *in vitro*. Also, massive eosinophilia was observed in approximately 70% of biopsy specimens from those non-IgE GI FA patients. However, it is still unclear how lymphocytes are recruited to the GI tract in those patients and to the skin in IgE-mediated food allergy patients. We examined plasma cytokine/chemokine levels in order to elucidate the Type 2-predominant immunopathogenesis shared by non-IgE GI FA and IgE-mediated milk allergy, and to identify differences in lymphocyte recruiting factors between each type of food allergies.

Methods: We recruited 28 pediatric non-IgE GI FA patients together with 17 pediatric IgE-mediated milk allergy patients, and obtained written informed consent. Plasma samples were collected before the resolution of the GI symptoms. Plasma cytokine/chemokine levels were measured by multiplex assay. This study was approved by the regional Ethics Committees.

Results: Similar levels of plasma Type 2 cytokines (IL-5 and IL-13), IL-17, as well as inflammatory cytokines/chemokines (IL-1β, TNF-α, IL-6, IL-8/CXCL8) were observed in either of the groups. Interestingly, the I-309/CCL1 level was significantly higher in non-IgE GI FA compared with IgE milk allergy, whereas the MIP-1β/CCL4 level was significantly lower.

Conclusions: Our results suggest that the mechanisms of lymphocyte recruitment in non-IgE GI FA may be distinct from those in IgE-mediated allergy, although both food allergies share a similar Type 2-predominant immunopathogenesis. Further studies are required to elucidate the molecular and cellular mechanisms underlying the recruitment of lymphocytes to, and the release of these chemokines from affected tissues.

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Biomarkers in Nasopharyngeal Aspirates at First Wheezing Episode to Predict Recurrent Wheezing

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Background: No biomarkers for predicting recurrent wheezing have been identified. To identify the biomarkers for the prediction of recurrent wheezing, we measured a broad panel of inflammatory mediators in the nasopharyngeal aspirates of infants who were followed up for 2.5 years after their first wheezing episode.

Methods: We enrolled 82 first-wheezing-episode infants (median age, 5.0 months) hospitalized for acute lower respiratory illness between August 2009 and June 2012 and followed these patients for 2.5 years. Nasopharyngeal aspirates and blood samples were obtained on the first day of hospitalization. Viral genomes were identified using RT-PCR and sequencing for 70 patients. The levels of 33 cytokines, tryptase, IgE, anti-RSV IgE, and anti-RSV IgG in nasopharyngeal aspirates were measured using enzyme-linked immunosorbent assays or Bio-Plex multiplex assay. Predictors of recurrent wheezing were examined using a stepwise logistic regression model with backward elimination.

Results: Sixty percent of the patients experienced recurrent wheezing episodes. In the nasopharynx of 93% of the first-wheezing-episode patients, one or more viruses were detected. The IFN-γ, Interleukin(IL)-2, IL-9, MIP-1α, and MIP-1β levels were significantly higher with recurrent wheezing than among patients without recurrent wheezing ($P < 0.05$ or 0.01). IL-10 level was significantly higher in patients detected RSV alone than HRV alone or RSV+HRV co-infection (P=0.049 Kruskal-Wallis test). However, no significant differences in the recurrent wheezing rates were observed between patients who tested positive for HRV and negative for HRV in the nasopharynx or

between those who tested positive for RSV and negative for RSV. The stepwise model demonstrated that the log MIP-1α level (OR, 7.72; 95% CI, 1.50 – 39.77; *P* = 0.015) was the strongest independent predictor of the occurrence of recurrent wheezing.

Conclusions: The measurement of MIP-1α levels in nasopharyngeal aspirates from first-wheezing-episode patients with acute lower respiratory illness may be useful for predicting recurrent wheezing and initiating early intervention. Although RSV infection might induce anti-inflammatory cytokine, IL-10 more than HRV infection, the association between respiratory infections in early life and recurrent wheezing was independent of virus type.

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Towards the identification of IgE antibody binding epitopes in Group 1 mite allergens

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Rationale: The analysis of antigenic determinants involved in IgE antibody binding to the group 1 dust mite allergens will allow the design of modified allergens, with reduced side-effects due to IgE cross-linking, for immunotherapy.

Methods: The X-ray crystal structures of three murine monoclonal antibodies (mAb) that interfere with IgE antibody binding were determined at high resolution (1.9-3Å), and the epitopes were analyzed by site-directed mutagenesis. Combinatorial libraries were constructed from the IgE repertoire of mite allergic patients, displayed on filamentous phage, and used to isolate Der p 1-specific single-chain variable fragments (scFv). A IgE scFv was expressed in yeast *Pichia pastoris* and purified by affinity chromatography. IgE antibody constructs were compared to the mAb of known specificity by immunoassays.

Results: The cross-reactive mAb 4C1 binds Der p 1 and Der f 1, whereas mAbs 10B9 and 5H8 are Der p 1-specific. A Der p 1-specific epitope for mAb 10B9 was engineered in Der f 1 by mutagenesis, and this analysis showed that the main residues involved in antibody binding differ for mAb 10B9 and 4C1, despite more than 70% overlap between both epitopes. These residues bound mAb 10B9 through different CDRs than CDR3. Two anti-Der p 1 IgE scFv were isolated from combinatorial libraries and showed different epitope specificity. A soluble Der p 1-specific scFv interfered with mAb 4C1 binding, but did not recognize the homolog Der f 1, similarly to the behavior of the Der p 1-specific mAb 10B9.

Conclusion: Recombinant IgE from combinatorial libraries and the X-ray crystal structures of allergen-antibody complexes provide a detailed analysis of antigenic determinants for the design of immunotherapy, in the context of the human IgE antibody repertoire.

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Isoallergen distribution in affinity-purified natural dust mite allergens

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BACKGROUND: Availability of highly purified, well-characterized allergens, which can be used as molecular reference materials is important for standardization of allergen extracts, determination of potency of allergy vaccines and validation of IgE molecular diagnostics. The objective of this study was to assess relative purity levels and isoallergen distribution of affinity-purified mite allergens Der p 1 and Der p 2.

METHODS: Isoform distribution of Der p 1 and Der p 2 in mite culture, during bioprocessing and in the purified allergen preparations was compared by LC-MS/MS. Data from the digests were analyzed against individual Der p 1 or Der p 2 isoform sequences.

RESULTS: Patterns of Der p 1 and Der p 2 isoforms were identical in mite culture and purified allergens. Based on diagnostic peptides, three isoforms of Der p 1 (0101, 0102 and 0124) were unambigously identified, the presence of 19 Der p 1 isoforms could be excluded. Der p 2.0110 (43%) and Der p 2.0101 (37%) represented the main Der p 2 isoforms. Purified Der p 1 and Der p 2 samples were free of contaminants.

CONCLUSIONS: Affinity-purification of natural Der p 1 and Der p 2 does not affect the original isoform distribution found in mite culture. Bioprocessing pathways have been established to yield high purity mite allergens with homogenous isoform profiles. Purified natural Der p 1 and Der p 2 can be used as molecular reference materials for allergen standardization. The limited number of isoforms present in mite culture has important implications for vaccines used for dust mite immunotherapy.

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Are dust mite allergens more abundant or more stable than other *Dermatophagoides pteronyssinus* proteins?

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Background: Understanding the characteristics that differentiate allergens from non-allergens remains a perplexing question in allergy research. General features such as protein stability and abundance from source are frequently cited as commonalities of allergens. However, rigorous statistical comparisons of allergens versus non-allergens on genomic and proteomic scales are lacking.

Methods: In this study RNA-seq methods were evaluated to assess the abundance of all proteins in the house dust mite *Dermatophagoides pteronyssinus* (DP). The thermodynamic stability of ~900 proteins and 21 allergens were evaluated using a combined chemical denaturation and mass spectrometry-based proteomics approach. Mass spectrometry was used to identify proteins in house dust extract.

Results: While the allergen transcripts are generally more abundant than the norm, many other proteins are more abundant. The allergens trend toward higher stability than the norm, but this is not a predictive characteristic. However, when abundance and stability are considered in combination, the allergens are statistically different from other DP proteins. The combination of abundance and stability for major allergens like Der p 1, Der p 2, and Der p 23 is rare (<3% of all proteins) but not unique. Extrapolation to the entire proteome of 26,992 proteins would suggest that there are likely ~800 proteins with similar biochemical characteristics. In order to better compare dust mite allergens and non-allergens in terms of human exposure, a mass spectrometry study to identify mite proteins in house dust extract was initiated. Group 1 and 2 allergens were identified as well as more than 15 non-allergens.

Conclusions: While DP allergens tend to be abundant *and* stable, other factors are likely required for sensitization. The finding of non-allergens from mites in house dust could imply that adjuvants in house dust may not promote all mite proteins to become allergens and suggests that allergens are an exclusive subset of mite proteins with the propensity to skew immune responses toward allergy. Further studies are needed to accurately quantify the relative amounts of allergens to non-allergens in house dust to better substantiate this conclusion.

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Lipophilic allergens: An underestimated risk in lupine?

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Background: Lipophilic allergens have been found to be triggers for severe clinical reactions. However, many of them are underrepresented in diagnostic extracts so far or are still unknown. Recently, we identified lipophilic allergens in the fat matrix of legumes (e.g. peanut). Although, the legume lupine is not so rich in lipids, it seems plausible that lupine also contains hydrophobic/lipophilic allergens, which have not been discovered yet. Therefore, identification and characterization of these allergens is necessary to close the existing diagnostic gap.

Objective: To identify new lupine allergens with a focus on hydrophobic/lipophilic and low molecular weight (LMW) proteins.

Material and Methods: Acidic extracts and isolated oil bodies from L. angustifolius and L. luteus were examined by SDS-PAGE. The proteins of interest were separated by gel filtration, and selected fractions were further purified by ion exchange chromatography. The purified proteins were screened for IgE binding by western blotting using sera of lupine and peanut allergic patients. IgE-reactive LMW components underwent N-terminal sequencing for identification, and databases were queried for homologous legume allergens. In addition, sensitization profiles of lupine and peanut allergic patients were obtained via CAP-FEIA.

Results: Focussing on LMW proteins, a 10 kDa component was isolated. Using N- terminal sequencing followed by a homology search against an expressed sequence tag database the protein was identified as a lupine lipid transfer protein (LTP) in L. luteus and L. angustifolius. Its IgE reactivity was demonstrated by immunoblot analysis. Additional low molecular weight proteins were isolated and **identified by a polyclonal anti-peanut oleosin antibody.**

Conclusions: Along with lupine LTP, there is some evidence for the presence of oleosins in lupine seed. As routine-allergy diagnostic tests are based on aqueous extracts, allergens that are predominantly present in lipid fractions of allergen sources remain "hidden" to the attention of allergists and consumers. Since cross- reactivity between lupine and other legumes (particularly peanut) is well known, hydrophobic/lipophilic lupine allergens might be a potential risk for legume-allergic patients.

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Structural, Functional and Immunologic Characterization of Profilins from Ragweed and Mugwort

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Rationale: Members of the profilin family have highly conserved sequences and are responsible for many cross-reactions between different sources of pollen and food allergens. The goal of this analysis is to characterize profilins originating from mugwort (Art v 4) and ragweed (Amb a 8) and compare them with birch profilin (Bet v 2).

Methods: Both Amb a 8 and Art v 4 were produced in a recombinant form. The proteins were characterized using X-ray diffraction analysis, thermal shift assays, gel filtration and dynamic light scattering. ELISA was used to determine whether the recombinant allergens were recognized by patients' sera.

Results: Milligram quantities of recombinant Amb a 8, Art v 4 and Bet v 2 were produced. All proteins were properly folded and were recognized by patients IgE. Crystal structure analysis revealed that the both Amb a 8 and Art v 4 share the same overall fold and are structurally similar to Bet v 2. Amb a 8, Art v 4 and Bet v 2 display relatively low thermal stability.

Conclusions: Recombinant Amb a 8, Art v 4 and Bet v 2 were successfully produced in *E. coli*. This comparison of molecular properties of Amb a 8, Art v 4 and Bet v 2 confirmed a high degree of structure conservation among analyzed pollen profilins. The structure of Amb a 8 is the first structure of a ragweed allergen that was experimentally determined. Sequence and structure conservation, as well as the propensity of profilins to oligomerize, seem to be crucial for triggering IgE-dependent inflammatory response.

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The novel cat lipocalin Fel d 7 and its cross-reactivity with the dog lipocalin Can f 1: Structural characterization and allergenic activity

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Background: Allergy to pets like cat (Felis domesticus) and dog (Canis familiaris) is the second most common indoor allergy in the western world and many patients are co-sensitized to cat and dog, suggesting allergenic cross-reactivity between these animals. The allergens Fel d 7 from cat and Can f 1 from dog are lipocalins that have high sequence identity (63%) indicating potential cross-reactivity. We have for the first time investigated the prevalence of sensitization to Fel d 7 in a European cat- sensitized population and elucidated its allergenicity and cross-reactivity with Can f 1 on an epitope level.

Methods: Sera from 94 Swedish cat dander-sensitized patients (as determined by ImmunoCAP e1; Phadia/ThermoFisher) were screened for IgE reactivity to Fel d 7 and Can f 1 by ELISA. IgE cross-reactivity was then determined by inhibition ELISA and allergenic activity of Fel d 7 was evaluated using a basophil activation assay. Structural analysis was performed with circular dichroism and Can f 1-peptide antisera were used to identify cross-reacting epitopes on Fel d 7.

Results: Thirty-seven (39.4%) cat-sensitized patients had IgE to Fel d 7 of whom 33 (89.2%) also had IgE to Can f 1. A moderate correlation was noted between IgE levels to Fel d 7 and Can f 1. The allergenic activity of Fel d 7 in cat allergic patients was confirmed by basophil activation assay. However, Can f 1 could only stimulate degranulation of basophils from patients that were co-sensitized to Can f 1. Nonetheless, inhibition studies demonstrated IgE cross-reactivity between Fel d 7 and Can f 1 and shared epitopes were revealed using Can f 1 peptide-specific IgG antibodies.

Conclusions: Fel d 7 is a frequently recognized allergen in a European cat-sensitized population that can be added to the list of important cat allergens. It has similar structure, shares epitopes and cross-reacts with the major dog allergen Can f 1. Fel d 7 may contribute to symptoms in cat and dog allergic patients by IgE cross- reactivity with Can f 1.

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Metabolomic analysis of aqueous pollen extract by NMR

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Background: Sensitization to allergens requires various cofactors that can include bacterial components such as lipopolysaccharide and flagellin. Other small molecules such as lipids and adenosine have also been shown to regulate dendritic cell responses.

Method: In this study, the concentration of small organic molecules in aqueous pollen extracts from birch, oak, Timothy grass, Bermuda grass, and ragweed pollens was measured with 1D-NMR spectroscopy.

Results: A principal component analysis of the metabolite profiles revealed high abundance compounds that could be used to differentiate the source pollen. For example, only Timothy and Bermuda grass pollen contain myo-inositol, while Bermuda grass and ragweed contain betaine but Timothy grass and tree pollens do not. In addition, the concentration of adenosine ranged from 20-60 uM in 10 mg of pollen/ 1 ml extract, similar to that determined previously by mass spectroscopy and high performance liquid chromatography. The concentration was highest in trees, followed by grasses, and ragweed.

Conclusions: The utility of this analysis is demonstrated by using the concentration of sugars, amino acids, and other compounds to identify pollen from an unknown source and classify mixtures with reasonable accuracy. Besides measuring the concentration of adenosine in aqueous pollen extract, the methodology is being tuned to quantify the lipid content in Folch extractions of pollens. Various lipids have also been implicated in skewing the immune response towards allergy.

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Clonal analysis of antibody repertoires during treatment with fast-dissolving grass sublingual allergy tablet

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Rational: Treatment with allergen-specific immunotherapy (AIT) tablets leads to specific serological changes. Functional studies indicate a transient induction of pre-existing allergen-specific IgE+ B cells and a functional relationship between IgE and IgG4 repertoires. The underlying changes in B cell repertoires caused by AIT, i.e. at the clonal level, are not known.

Methods: Recent advances in sequencing of entire antibody variable gene repertoires present in biological samples allow for a detailed analysis of the dynamic behavior of B cell repertoires. Total RNA was purified from 10^7 PBMCs and used as template for variable gene sequencing. Annotation of antibody attributes of raw reads was done using a newly developed bioinformatics pipeline, which, among others, assigns IgH-isotype, VH CDR3 sequence (clonotype), germ line VH-gene usage, and a list of somatic mutations to each read.

Results: A total of 28 blood samples obtained from healthy and atopic subjects have been sequenced. On average, $1.3x10^6$ sequencing reads per sample lead to on average 103.948 unique VH CDR3 sequences after annotation. This indicated good repertoire coverage considering that each samples contained roughly 10^6 B cells. We further evaluated coverage by sequencing of technical duplicates and by sequencing of samples from the same subject taken at different time points. Technical duplicates showed high degree of repertoire overlap of up to 85% in highly frequent reads. Repertoire overlap between samples from the same subject at different time points was significantly larger than the overlap between independent subjects, indicating some degree of conservation of the antibody repertoire over time. For all PBMC samples it was found that low frequency reads were mainly of the IgM isotype whereas more frequent reads progressively shifted towards more mature IgH-isotypes. This agrees well with the expected shift in antibody IgH-isotypes during clonal selection and expansion.

Conclusions: The data confirmed the robustness of the methods and thereby allows for identification of disease and treatment relevant antibody sequences directly from the blood of atopic subjects. We aim to establish a detailed time course of the early B-cell response in order to characterize local and peripheral B cell repertoires in patients undergoing AIT.

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Macrophage secrete discrete amount of cortisol, which can be captured by the lipocalin-allergen Bos d 5 , thereby influencing LCN2 expression

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Background: We previously showed that lipocalin-allergens, like the human lipocalin-2 (LCN2), are able to bind to iron-siderophore complexes. Macrophages are not only a prominent source of LCN2, but also of bioactive agents such as cortisol. Moreover, the expression of LCN2 is affected by cortisol due to a glucocorticoid response element in its promoter region. Here, we investigated whether cortisol-iron complexes released by macrophages could be bound by the lipocalin allergen Bos d 5, which might explain its immune-skewing properties in milk allergy.

Methods: Sera of healthy and allergic individuals (n=160) were tested for LCN2 concentrations. In silico analyses were conducted with Bos d 5 and cortisol-iron complexes using Autodock Vina. UV-VIS spectra and Prussian blue staining of Bos d 5 with iron-siderophore complexes were taken in vitro. Alternatively-activated macrophages were generated from human blood and cultured in the presence and absence of apoBos d 5. Supernatants were analysed for cortisol and lipocalin-2 content by ELISA.

Results: Allergics have significantly lower LCN2 levels than non-allergic individuals. Calculated affinities of cortisol to Bos d 5 reached the nanomolar range upon virtual addition of iron. Binding of iron to cortisol could be confirmed by Prussian blue staining and UV-VIS spectral analyses. Alternativ-activated M2b-macrophages secreted cortisol and LCN2, whereas hardly no LCN2 was detected in M2a or M2c. Addition of apoBos d 5 to M2b- macrophages resulted in decreased levels of free cortisol and LCN2.

Conclusion: We show for the first time that allergics have lower levels of LCN2 and that a lipocalin-allergen Bos d 5 may bind cortisol-iron complexes released by macrophages. The buffering capacity of Bos d 5 for free cortisol may disturb the immune balance in allergics.

Supported by the F4606-B19 of the Austrian Science Fund FWF.

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Conformational IgE-binding Epitopes of Ara h 2 and Ara h 6

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Rationale. IgE-mediated immediate hypersensitivity reactions occur when an allergen effectively cross-links IgE/FcεR1 complexes on the surface of mast cells and basophils. We hypothesize that identification of the key IgE-binding conformational epitopes of the most potent peanut (PN) allergens, Ara h 2 and Ara h 6, will lead to novel diagnostic and therapeutic approaches.

Methods. Phage: We screened a phage peptide library (12-mer peptides) with affinity-purified IgE from sera of 4 PN allergic patients. The phages were subjected to biopanning with anti-PN IgE, negative selection, and then two further rounds of biopanning. Binding to anti-PN IgE was confirmed by ELISA. Mimotopes were sequenced. Modeling: Mimotopes were grouped according to the amino acid composition and distribution. The EpiSearch program (http://curie.utmb.edu/episearch.html) was used to determine areas of the 3D-structures of Ara h 2 and Ara h 6 with similar amino acid composition to that of the mimotope groups. The similarity was measured by comparing the physical-chemical properties of the residues in the input sequences (mimotopes) to those of potential surface patches. Candidate patches were then ranked by counting matching residues (*i.e.* pairs of residues in the input sequence and in the surface patch with a property distance (PD) value of less than 8).

Results: Three groups of similar mimotopes were defined and related to two surface exposed regions (A and B) in helices III and IV of Ara h 2. These patches includes parts of previously identified linear epitopes (A: epitopes 5, 6, and 7; and B: epitopes 2 and 4) as well as amino acids that are not part of previously identified linear epitopes (Otsu et al. Clin. Exp. Allergy 2014: 45: 471-484). Similar surface exposed regions were found to occur in structurally equivalent positions on Ara h 6.

Conclusions: We identified 15 novel mimotopes that map to 2 patches on the surface of Ara h 2 and Ara h 6, representing conformational epitopes. We hypothesize that these conformational epitopes will prove to be critical for cross-linking of IgE/FcεR1.

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Molecular analysis of IgE antibody responses to shrimp allergens in patients from different geographic regions

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Background: Shrimp is a common cause of severe food allergic reactions worldwide. The aim of this preliminary study was to examine IgE antibody responses to northern shrimp (*Pandalus borealis*) and giant tiger prawn (*Penaeus monodon*), to cephalothorax and tail muscle separately and to individual allergen components in sera of shrimp allergic subjects from different parts of the world.

Methods: Sera from 79 shrimp allergic European, North American and Japanese subjects were studied. IgE levels to extracts and recombinant Pen a 1 (tropomyosin), Pen m 3 (myosin light chain 2), Pen m 4 (sarcoplasmic Ca-binding protein) and Pen m 6 (troponin C) and natural Pen m 2 (arginine kinase) were analysed by ImmunoCAP. IgE levels ≥0.35 kU_A/L were considered positive.

Results: 63 of the 79 subjects (80%) tested positive to either or both shrimp species studied. The frequency of sensitisation to rPen a 1, nPen m 2, rPen m 3, rPen m 4 and rPen m 6 were 33%, 29%, 18%, 9% and 20%, respectively. Large regional differences in frequency of sensitization were observed for some components. In the Japanese subjects, nPen m 2 was the most frequently recognised component (12/22, 55%), in contrast to only 5/30 (17%) among the South European subjects. Conversely, the most prevalent sensitizations among the latter subjects were to rPen a 1 (12/30, 40%) and rPen m 6 (10/30, 33%) while only 5 (23%) and 2 (9%) of the 22 Japanese subjects were sensitized to the same components. Among the North European subjects, sensitization to Pen m 2, Pen m 3 and Pen m 4 was present in 13-17% while IgE to Pen a 1 was present in 6/23 (26%). In a majority of all subjects, the combined component responses were comparable to or greater than that to *P. monodon* extract. Many subjects testing negative to all individual components showed a higher IgE response to shrimp cephalothorax than to tail muscle.

Conclusion: Diverse and regionally different sensitization patterns to shrimp allergens were observed. In a subset of subjects, the IgE response to *P. monodon* extract could not be accounted for by any of the components studied.

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Histamine release from passively sensitized human basophils and the impact of allergen specific IgE

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Background: In the diagnosis of allergy, the basophil histamine release (HR) test can be used in addition to the conventional measurement of allergen-specific IgE. The use of passive sensitization of basophils increases the versatility of the method and allows for comparison of different sera unbiased by interference from the cellular component which is appropriate when performing allergen characterisation. However, some patient sera perform poorly in the passive sensitization HR test. Therefore, we investigated how the level and percentage (compared to total IgE) of specific IgE, affects the HR response using Bet v 1 and serum from birch pollen sensitized patients as a model system.

Methods: Sera with birch pollen specific IgE (40 sera, 10 from CAP class 1 to 4+) and a negative control serum pool were used to passively sensitize donor basophils, obtained from buffy coat blood (n = 3). The cells were then incubated (30 min) with birch allergen Bet v 1 (0.2 – 50 ng/mL) or PMA/ionomycin mixture (maximal HR) and released histamine was quantified fluorometrically. Results are given as HR% of maximal release.

Results: We found that a level of birch pollen specific IgE > 3.51 kU_A/L (CAP class 3 and 4+) gave a significant reproducible HR: 60/60 vs. 5/30 in CAP class 1 and 21/30 in CAP class 2. The HR% correlated with the CAP class but within the classes, the absolute amount of specific IgE was less well related to HR. Thus, we found sera with almost identical specific IgE levels to give different HR responses and, likewise, sera with three-fold difference in specific IgE level to give HR responses of the same magnitude. The dissociation between absolute amount of specific IgE and HR could not be explained by the percentage of specific IgE.

Conclusion: The results demonstrate that the IgE titer is strikingly robust in predicting the ability to sensitize basophils and produce a measurable HR. This suggest that by proper selection of mid to high titer sera, the passive sensitization HR test represent a consistent system for studies and characterisation of the biological potency of allergens.

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A Randomized Controlled Trial of Intradermal Grass Pollen Immunotherapy For Seasonal Allergic Rhinitis

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Background: Subcutaneous and sublingual immunotherapy with high dose grass pollen extract inhibits allergen-induced cutaneous late phase responses and is clinically effective. We previously reported that 6 repeated intradermal injections of grass pollen (nanograms of allergen) also profoundly suppresses allergen-induced cutaneous responses (Rotiroti et al., J Allergy Clin Immunol 2012 Oct;130(4):918-24). In this study, we conducted a phase II randomized double-blind placebo-controlled trial to evaluate the efficacy and safety of grass pollen intradermal immunotherapy (IDIT) for treatment of allergic rhinitis.

Methods: We randomly assigned 93 adults with grass pollen allergic rhinitis to receive 7 pre-seasonal IDIT injections (each containing equivalent of 7 ng of Phl p 5 major allergen) or histamine control. The primary end point was combined daily symptom-medication scores (CSMS) during the 2013 pollen season. Sera were collected for antibody measurements and skin biopsies were taken after the pollen season following an intradermal skin allergen challenge for T cell culture. Cutaneous late responses were measured 4 and either 7, 10 or 13 months post-IDIT.

Results: No significant difference in the primary endpoint (CSMS) was observed between treatment arms (median difference, 14; 95% confidence interval [CI], -172.5 to 215.1; P=0.80). Paradoxically, amongst secondary endpoints, nasal symptoms measured with daily scores (median difference, 35; 95% CI, 4.0 to 67.5; P=0.03) and visual analogue scale (median difference, 53; 95% CI, -11.6 to 125.2; P=0.05) were higher in the IDIT group. IDIT was associated with relative increases in Phl p-, Phl p 1- and Phl p 5-specific IgE (all P≤0.001) compared to the control arm. T cells cultured from skin biopsies of IDIT subjects showed higher and lower expression of surface Th2 marker CRTH2 (P=0.04) and Th1 marker CXCR3 (P=0.01), respectively. IL-5 mRNA detected by microarray was more highly expressed by skin T cells derived from IDIT participants (P=0.03). Late responses to grass pollen were still inhibited after IDIT up to 7 months (P=0.025) but not at 10-13 months.

Conclusions: Although grass pollen IDIT suppressed skin late responses, this treatment was not clinically effective but resulted in immunological priming and worsening of allergic rhinitis symptoms.

Acknowledgement: This project is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership.

Disclaimer: The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NHS, NIHR or the Department of Health.

*The EME Programme is funded by the MRC and NIHR, with contributions from the CSO in Scotland and NISCHR in Wales and the HSC R&D Division, Public Health Agency in Northern Ireland.

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Comparison of Relative and Absolute Treatment Differences Between Sublingual Immunotherapy Tablets and Pharmacotherapies for Seasonal and Perennial Allergic Rhinitis: Pooled Analyses of Clinical Trials

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Background: No head-to-head trials have compared the treatment effects of sublingual immunotherapy (SLIT) tablets and pharmacotherapy for allergic rhinitis (AR). Indirect treatment effect comparisons are difficult because of methodological differences in trial design/conduct. These analyses compare the effect on nasal symptoms with SLIT-tablets vs selected pharmacotherapies for seasonal and perennial AR (SAR and PAR) as analyzed by standardized mean difference (SMD) or difference relative to placebo.

Methods: Data were compiled and pooled from clinical development programs for various AR treatments. A fixed-effect meta-analysis method estimated treatment differences in average total nasal symptom scores (TNSS). Subjects scored symptoms daily during entire pollen seasons in 6 Timothy grass SLIT-tablet trials (n=3,094) and 2 ragweed SLIT-tablet trials (n=658), and during the last 8 weeks of treatment in 2 house dust mite (HDM) SLIT-tablet trials (n=1,768). Subjects scored symptoms daily in 7 montelukast (10 mg; n=6,799), 9 desloratadine (5 mg; n=4,455), and 8 mometasone furoate nasal spray (MFNS; 200 mg QD; n=2,140) SAR or PAR trials. Symptom scoring for pharmacotherapy was ≈2 weeks for SAR and 4–6 weeks for PAR.

Results: In grass and ragweed SLIT-tablet trials, the relative average improvement in TNSS versus placebo was 16.3% (SMD, –0.26) and 17.1% (SMD, –0.29), respectively. In the HDM SLIT-tablet trials, TNSS relative improvement versus placebo was 16.1% (SMD, –0.24). In the montelukast, desloratadine, and MFNS trials, overall relative improvement in TNSS versus placebo was 5.4% (SMD, –0.18), 8.5% (SMD, –0.25), and 22.2% (SMD, –0.61), respectively, for SAR trials, and 3.7% (SMD, –0.12), 4.8% (SMD, –0.13), and 11.2% (SMD, –0.28) for PAR trials.

Conclusions: Based on relative effect vs placebo, grass and ragweed SLIT-tablets had a comparable effect on nasal symptoms vs MFNS, and were numerically superior to montelukast and desloratadine in SAR trials. HDM SLIT-tablet elicited similar improvement on nasal symptoms as SAR SLIT-tablets, and a similar or greater improvement than all the evaluated pharmacotherapies in PAR trials. SMDs indicated the same effect size as relative differences among pharmacotherapies, but underestimated the effect size for SLIT-tablets. The underestimation is likely due to trial design characteristics such as allowed rescue medication use by placebo patients in SLIT-tablet studies.

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Timothy grass pollen SLIT tablets' SPT potency compared to a US reference extract

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Background: To compare the relative potency in skin prick testing of solution and tablet extracts of Timothy grass pollen (TIM) of 2 European manufacturers (ALK-Abelló, Stallergènes), with an FDA approved extract (REF) of 10,000BAU/mL

Method: This is a prospective, multicenter, triple blinded, randomized study in which the *in vivo* extract potency was determined, based on the surface of the wheal size obtained in TIM allergic patients. The four tested TIM extracts were: Soluprick®, Staloral® 300IR, and Grazax® and Oralair® 300IR dissolved in 1mL 50% glycerin (under GMP standards). The SPTs were carried out in quadruplicate with the concentrate extracts and three serial half-log dilutions, and +/- controls. The study took place at study sites with different climatologic conditions. To determine if there exists a statistically significant difference between the relative potency of the TIM extracts a parallel line bioassay was carried out using the geometric mean of the surface of the four wheals of the SPTs per extract and per concentration (Wilcoxon, Asymp. Sig. (2-tailed)). Based on the corrected wheal sizes of the concentrate extracts in relation to the REF, BAU values were calculated.

Results: For the concentrated extracts and their serial dilutions an almost linear dose-response could be detected for all 4 extracts tested (with the exception of the wheals for the highest dilutions, R^2 for the lines ranged from 0.93-0.98): the wheal surfaces were (concentrate/dilution 1:3/dil 1:10/dil 1:30): Sol A: 1.54/1.06/0.59/0.17; Tabl A: 0.92/0.69/0.48/0.23; Sol B 1.80/1.18/0.81/0.19; Tabl B 1.25/0.83/0.49/0.18cm^2. As wheal surface, not diameter was used as the variable, no semi-log-transformation was necessary. Differences in wheal size between concentrate extracts reached statistical significance for all (p between 0.05 and 0.001), except Soluprick-REF. The calculated BAU compared to the REF values for both solutions were 10350 and 12086 BAU/mL and for the tablets 6133 and 8357 BAU.

Conclusion: Based on SPT whealsizes grass-tablet potency seems to be higher than the reported 2800BAU. There is a difference between the allergen concentration as measured in SPT of both tablets, indicating that probably other factor, apart from the exact allergen content are of importance for the efficacy of SLIT.

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Allergen-specific B cell responses to allergen-tolerance induction are characterized by expansion of B_R1 cells, IgG4-class switch recombination and CCR5 expression.

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Background: Allergen-specific immunotherapy (AIT) can induce immune tolerance to allergens. Although a lot has been reported on T cell and regulatory T cell responses, the investigation of circulating allergen-specific B cell and antibody responses remained to be elucidated.

Objective: To identify the role of allergen-specific B cells in allergen tolerance induction and to compare these responses between allergic patients receiving AIT and naturally exposed healthy beekeepers.

Methods: Bee venom allergic patients who received AIT and healthy beekeepers were used as two established allergen tolerance models. We identified circulating allergen-specific B cells by using phospholipase A2 (PLA) labeled with two fluorescent dyes. Flow cytometry was used to determine the expression of regulatory B cell-associated surface markers, interleukin-10, chemokine receptors and immunoglobulin heavy chain isotypes.

Results: A strikingly similar response was observed at the level of allergen-specific B cells was in bee venom allergic patients after AIT and in healthy beekeepers after multiple bee stings. This response was characterized by an increase of PLA-specific class-switched memory B cells, as well as $CD27^+CD38^{hi}$ plasmablasts increased after AIT and during beekeeping season. We also observed an increased frequency of IL-10 secreting $CD73^-CD25^+CD71^+$ B_R1 cells in both AIT patients and beekeepers. Furthermore, PLA-specific $IgG4^+$-switched, but not IgA- or IgG1-switched memory B cells expanded significantly after AIT in patients and after multiple bee stings in beekeepers. PLA-specific B cells purified after AIT or multiple bee stings also secreted higher levels of PLA-specific IgG4, while IgG1 levels remained equal. These observations were corroborated by PLA-specific serum immunoglobulin measurements. Finally, PLA-specific B cells showed increased CCR5 expression in both groups after high-dose bee venom exposure.

Conclusion: Allergen tolerance induction to bee venom is accompanied by expansion of allergen-specific B cells with a tolerogenic potential, characterized by IL-10, IgG4, and CCR5 expression. These findings support a functional immunoregulatory role for allergen-specific B cells in allergen tolerance induction. These findings can be investigated in other AIT models to determine whether they can serve as a biomarker of early and successful AIT response.

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MACROPHAGES SPECIFICALLY RELEASE CORTISOL AND IL-10 UNDER IgG4 STIMULATION; POSSIBLE EXPLANATION OF ALLERGEN IMMUNOTHERAPY MECHANISM.

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Background: Type I hypersensitivity is characterized by a Th2 polarization of specific and innate immune cells, resulting in an isotype switch to IgE and allergic inflammation. Allergen-specific immunotherapy (AIT) is the only curative therapy for it. In AIT, typically an increase of allergen specific "blocking" IgG4 and induction of T- and B-regulatory cells are observed. However, the quest for a clinically predictive biomarker is still ongoing.

We investigated here whether IgG4 could interact with macrophages. These innate cells are not only an important source of the immunomodulatory cytokine IL-10, but also of the anti-inflammatory hormone cortisol.

Methods: Primary macrophages from healthy PBMCs (MDMs), or the macrophage cell line THP-1 were first differentiated into a Th2 polarized activation state (M2) by treatment with M-CSF and LPS (subtype M2b) or IL-4/IL-13 (M2a), respectively, and cultured in the presence of plate-fixed IgG1 or IgG4, in an allergen-nonspecific manner. The supernatants were analyzed in radioimmunoassay or ELISA.

Results: We observed that only co-incubation of M2b with IgG4 significantly increased cortisol and IL-10 production, whereas IgG1 or medium alone had no such effect. Moreover, these effects were accompanied by a significant downregulation of CD14 and CD206 on the macrophages in flow cytometry.

Conclusion: We propose that our results add significantly to the understanding of AIT. We report here for the first time that IgG4, but not IgG1, via macrophages exerts a direct immune tolerizing effect based on the release of IL10 and cortisol. Considering the impact of cortisol in immunosuppression, we anticipate that the principle may have a predictive clinical value.

Supported by Austrian Science Fund FWF project SFB F4606-B19.

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T cell immunogenicity of major and minor grass pollen allergens

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Background: IgE mediated allergy to grass pollen has a high prevalence world-wide. Grass pollen is the most important outdoor allergen source causing allergic rhinitis and may also induce asthma in predisposed individuals. Investigations of the frequency of IgE responses to individual grass pollen allergens indicates that group 1 and group 5 allergens are the most frequently recognized allergenic proteins followed up by group 2, group 4 and group 3. T cell reactivity has mainly been investigated for group 1 and group 5 allergens and the current study aimed to determine the frequency of T cell responses to the individual natural purified group 1, 2, 3, 4 and 5 allergens from the temperate grass *Phleum pratense* (Phl p 1 to 5) and relate this to the IgE reactivity.

Methods: In this study we have investigated T cell immunogenicity of important grass allergens in more than 50 T-cell lines derived from Danish grass pollen allergic donors. Phl p grass allergen specific T-cell lines were established from donor derived peripheral blood mononuclear cells (PBMC) through stimulation with a mixture of natural purified grass allergens (Phl p 1, Phl p 2, Phl p 3, Phl p 4, and Phl p 5) *in vitro*. T-cell responses were studied by fluorospot (IL-5 and IFN-γ) and proliferation assays. In parallel, serum samples were used for measurement of IgE specific to the individual allergens by Immunocap as well as ISAC measurements.

Results: IL-5 and/or IFN-γ release and T cell proliferation assays show that the majority of the donors react to the Phl p 4 and 5, whereas responses to Phl p 1, 2 and 3 were less frequent. The data derived from these 50 Danish donors suggests that Phl p 4 is a strong T cell immunogen and Phl p 1 to be less important for the T-cell activation in grass allergic patients when compared to Phl p 5 and Phl p 4. In contrast, Phl p 1 and 5 were recognized by IgE from more than 80% of the patients whereas less than 50% were IgE sensitised towards Phl p 2 and 4.

Conclusion: Taken together the data on IgE and T cell reactivity will assist us in understanding the Immunogenicity of allergens and may help select individual allergens for novel Immunotherapy vaccines for grass allergy.

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Humanized mice as in vivo model for allergen-specific immunotherapy of IgE- mediated allergy

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Allergen-specific immunotherapy (AIT) is the only treatment improving IgE-mediated allergy with long-term benefit. Unfortunately, AIT fails in a substantial number of treated patients. Therefore, one major focus of allergy research is the development of allergy vaccines with improved efficacy and safety. To establish an in vivo model to study the efficacy of such vaccines in the allergic human immune system we sought to humanize NOD-SCID IL-2Rγc-/- (NSG) mice with PBMC from birch pollen-allergic patients. To this aim, PBMC were injected intraperitoneally (i.p.) together with birch pollen extract (BPE), followed by an i.p. boost with BPE after 7 days. From day 20-22

NSG-mice received either BPE or PBS intranasally (i.n.) and at day 24 their airway hyperresponsiveness (AHR) was evaluated by invasive measurement of airway resistance in response to increasing doses of methacholine. In parallel, bronchial alveolar fluid (BALF) was collected and investigated for inflammatory cell types by flow cytometry. Mice i.n. exposed to BPE showed higher numbers of murine basophils, eosinophils, granulozytes and macrophages and significantly increased AHR as compared to NSG-mice i.n. challenged with PBS. Human cells were detected in lung, spleen and BALF. In conclusion, NSG-mice were successfully humanized and showed signs of allergic lung inflammation. We are now in the process of employing this in vivo model to test novel allergy vaccines consisting of Bet v 1 fused to TLR-ligands developed in our laboratory.

Supported by the Austrian Science Fund, project SFB-F4610.

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Mast Cell Activation Induced by T cell – Derived Microvesicles: a possible role for miR 4443

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Background: We have recently shown that mast cells can be activated by microvesicles derived from activated T cells (mvT*) to degranulate and release several cytokines. These events are associated with RAS activation and sustained ERK phosphorylation. The aim of this study was to analyze the possible effect of microRNAs delivered by MVs on mast cell activation.

Methods: The high-throughput microRNA profiling was performed using NanoString technology platform and was validated by real time PCR. The biological role of mvT*- derived microRNA was verified by overexpression of these microRNAs in LAD2 cells using mimic molecules and analyzing their predicted targets.

Results: 6 microRNAs; miR-4443, miR-146a, miR-29, miR-210, miR-24 and miR-9 were found to be overexpressed in human mast cells stimulated with mvT*. In this work we focused on the biological role of miR-4443, that has not been recognized thus far in mast cells and is shown to be highly overexpressed in response to activation of mast cells with mvT*. MiR-4443 was also found to be expressed in activated T cells and in their microvesicles, indicating that these mvT* may deliver it into mast cells. One of the predicted targets of miR-4443 is the tyrosine phosphatase PTPRJ (CD148) that acts as down regulator of receptor tyrosine kinase activity and was found to be able to decrease the RAS-ERK signaling pathway. To test the ability of miR-4443 to regulate the 3'UTR of PTPRJ, we used the luciferase reporter assay. Co-transfection of mimics miR-4443 and plasmid containing the 3'UTR of PTPRJ, revealed a decrease of the luciferase gene level indicating that PTPRJ is indeed a target of miR-4443. Furthermore, stimulation of mast cells with mvT* resulted in decreased PTPRJ protein level leading to sustained ERK phosphorylation.

Conclusion: Stimulation of mast cells with mvT* leads to overexpression of miR-4443 that serves as PTPRJ negative regulator. This may explain, at least in part, the sustained ERK phosphorylation and mast activation in response to stimulation with mvT*. Thus, by carrying a cargo of genetic information from one cell type to another, MVs may play an important role in T cell - mediated inflammatory processes where mast cells were found to be involved.

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The mast cell-clock regulates blood histamine levels: implication for stress-induced exacerbation of allergy

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Background: Blood histamine levels show the circadian pattern in rodents and humans. However, the mechanisms and its pathological significance remain unknown.

Objective: We determined whether the molecular clock in mast cells played a role in circadian regulation of blood histamine levels and how this mechanism affected mast cell-dependent allergic reaction.

Methods: We examined the effects of mast cell-deficiency and genetic or environmental disruption of the mast cell clockwork on blood histamine levels and also on compound 48/80-induced plasma extravasation in mice.

Results: Blood histamine levels showed a time-of-day dependent variation in wild-type mice, which was absent in mast cell-deficient or mast cell-specific circadian gene *Clock*-mutated mice. *Clock* regulated expression of organic cation transporter (OCT)-3 which involved histamine release in mast cells in a circadian manner and OCT3 inhibition abolished daily variations in blood histamine levels. Mice suffering from restraint stress or housed under aberrant light/dark conditions resulted in disruption of the mast cell clockwork and loss of daily variations in OCT-3 expression and blood histamine levels. The extent of compound 48/80-induced plasma extravasation showed a time-of-day-dependent variation associated with blood histamine levels in wild-type mice, but not in *Clock*-mutated mice and in mice suffering from restraint stress, or housed under aberrant light/dark conditions.

Conclusion: The mast cell-clock mediates circadian regulation of blood histamine levels by temporally controlling OCT-3 expression. Accordingly, environmental stresses that disrupt the mast cell clockwork resulted in the absence of the daily variations in OCT-3 expression, blood histamine levels, and mast cell-dependent allergic reaction, which may be associated with stress-induced exacerbation of allergic reaction.

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A chimeric protein containing the C-terminus of Bet v 1 is a potent inducer of basophil degranulation

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Background: Characterization of IgE epitopes of the major birch pollen allergen, Bet v 1, is essential for the design of hypoallergenic vaccine components. We showed that the Bet v 1-specific IgE response is polyclonal and highly patient-specific and that important IgE epitopes are distributed across the whole surface of Bet v 1. However, little is known about the biologic relevance of IgE specific for different areas of the Bet v 1 surface.

Methods: Four non-overlapping contiguous Bet v 1-specific surface areas were grafted onto the Bet v 1-related celery allergen Api g 1. The resulting chimeras, called Api-Bet-1 to Api-Bet-4, were expressed in *Escherichia coli* and purified by standard chromatographic methods. The aggregation behavior of Bet v 1, Api g 1 and the chimeras was analyzed by dynamic light scattering. Basophil activation tests using whole blood of 15 birch pollen allergic donors were performed with different concentrations (10 pg/mL–1 µg/mL) of Bet v 1, Api g 1 or the chimeric proteins. The percentage of CD63-positive cells in the $CCR3^{+}CD123^{+}$ population was measured by flow cytometry. The concentration required to reach half maximum activation was defined as AC50.

Results: Dynamic light scattering revealed that the recombinant proteins were monomers. Bet v 1 exhibited the highest potency to activate basophils with a median AC50 of 0.2 ng/mL. The scaffold protein Api g 1 showed a median AC50 of 30 ng/mL. Api-Bet-3 revealed the lowest median AC50 among the chimeras at 2.2 ng/mL compared to Api-Bet-2, Api-Bet-1 and Api-Bet-4 with AC50s of 3.5 ng/mL, 4 ng/mL, and 6 ng/mL, respectively. 2/15 patients (13%) showed basophil activation exclusively after stimulation with Bet v 1. In 6/13 patients who reacted with the chimeras (46%), Api-Bet-3 showed the highest ability to activate basophils.

Conclusion: Grafting of four non-overlapping Bet v 1-specific areas onto Api g 1 increased its potency to activate basophils. This reveals that the whole surface area of Bet v 1 contains biologically active IgE-binding epitopes. However, Api-Bet-3 was the most potent chimera to activate basophils, indicating that important IgE-binding epitopes are located at the C-terminus of Bet v 1. The study was supported by the Austrian Science Fund (FWF) grants SFB F4608 and F4610 and by the Christian Doppler Laboratory for Immunomodulation.

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Activation of Human Basophils by Epithelial Cells: A Role for IgE Interaction with Galectin-3

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Background: Mouse models stress the importance of epithelial cell (EC)-derived cytokines, such as TSLP, in activating basophil IL-4 secretion required for sensitization to allergens. Evidence for these cytokines activating human basophils has been less forthcoming using *in vitro* models. To investigate this discrepancy, we hypothesized a requirement for direct interaction with ECs and/or with cytokines/proteins other than TSLP in activating human basophils. **Method:** Basophils (>99% purity), in medium alone or with IL-3±anti-IgE, were co-incubated with recombinant human (rh)TSLP, rhIL-33, or rhIL-25. Analogous experiments co-cultured basophils (1-72h) directly with EC lines (A549 and BES2B), to allow cell-to-cell contact. Supernatants were concurrently tested for histamine [automated fluorometry] and cytokines [ELISA]. Antibodies targeting cytokine receptors and cell surface markers were evaluated in neutralization experiments. Lactic acid (pH 3.9) treatment combined with passive sensitization tested the role of IgE. **Results:** rhIL-33 (but not rhTSLP or rhIL-25) augmented IL-13 secretion (9-fold) from basophils co-treated with IL-3 (n=4, P=0.03), with minimal effects on histamine and IL-4. For comparison, basophils additionally released histamine and marked levels of IL-4 when co-cultured with A549 EC in the presence of IL-3, but without allergen or IgE cross-linking stimuli. IL-4/IL-13 levels were ~10-fold greater than in control cultures without EC (p=0.002, n=12). The inability to detect IL-33/TSLP, or to neutralize activity possibly mediated by these cytokines, suggested a unique mode of basophil activation by ECs. Indeed, the half-maximal rates for histamine (4h) and IL-4 (5h) secretion were strikingly slower than observed with standard IgE-dependent activation. However, immunoglobulin stripping combined with passive sensitization ± omalizumab showed a clear dependency for basophil-bound IgE. This was substantiated by requirement for cell-to-cell contact, as assessed using trans-well cultures. In exploring a role for IgE-binding proteins, A549 ECs were found to express galectin-3. N-acetyllactosamine, which is reported to block IgE/galectin-3 interactions, significantly suppressed basophil responses augmented in co-culture with ECs. Finally, galectin-3 protein bound to microtiter wells mediated a similar activation of basophils whereas soluble galectin-3 did not. **Conclusions:** We predict that ECs expressing galectin-3 have the capacity to activate basophils for mediator release and IL-4/IL-13 secretion by inducing a non-specific IgE cross-linking independent of allergen.

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Role of kinases, dynamin and the cytoskeleton in regulating Siglec-8 engagement-induced endocytosis, reactive oxygen species (ROS) production and apoptosis in primary human eosinophils

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Eosinophils are major effector cells in allergic disease. On their surface they express sialic acid-binding immunoglobulin-like lectin (Siglec)-8 that induces their apoptosis. Hence, Siglec-8 could be a useful target for monoclonal antibody- and glycan ligand-based therapies for diseases involving these cells. Effective delivery of therapeutic agents to these cells requires an understanding of the dynamics of Siglec-8 surface expression, which have not been elucidated. Furthermore, while Siglec-8 binding causes apoptosis through increased mitochondrial damage and ROS production, its exact signaling mechanisms are unknown.

Siglec-8 engagement with the monoclonal antibody (2C4) or a specific synthetic ligand (6′-O-sulfo-3′-sLacNAc-PAA) led to Siglec-8 endocytosis in IL-5-activated primary eosinophils that proceeded slowly, with about half of the surface pool of Siglec-8 being internalized in 90 minutes. This process was prevented by treatment with disruptors of actin cytoskeletal dynamics (latrunculin B and jasplakinolide), a broad inhibitor of tyrosine kinases (genistein), or a dynamin inhibitor (dynasore), but not by an inhibitor of microtubule assembly (nocodazole) or a Src family kinase inhibitor (PP1). Using the same antibody to induce apoptosis, in combination with candidate signaling molecule inhibitors, we observed that monoclonal antibody 2C4-mediated eosinophil apoptosis was blocked with inhibitors of Src family kinases (PP1 and SU6656), Btk (ibrutinib), PI3K (LY294002), PKC (GF109203x), and protein tyrosine phosphatases (sodium orthovanadate) at IC_{50}s of 4.5 µM, 1.7 µM, 0.9 nM, 1.3 µM, 2.5 µM, and 22 µM, respectively. In parallel, complete inhibition of ROS production occurred at the expected IC_{90}s of PP1, ibrutinib, and LY294002, but not SU6656 or GF109203x. Western blot analysis following Siglec-8 cross-linking with 2C4 showed increased phosphorylation of Src_{530}, PI3Kδ, Blk, and Csk that was detectable within 15 min, while increased phosphorylation of c-Abl was detectable by 60 mins.

In summary, specific subsets of tyrosine kinases appear to be involved in Siglec-8–mediated ROS production and apoptosis in human eosinophils, while Siglec-8 internalization involves tyrosine kinases, dynamin and the actin cytoskeleton. Besides antibody targeting, the dynamics of Siglec-8 internalization appear to be suitable for sustained targeting and may allow for the delivery of drugs selectively into eosinophil intracellular compartments to treat diseases involving these cells.

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Eosinophil cytolysis occurs through necroptosis

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Background: Eosinophils are a subset of granulocytes involved in the pathogenesis of different diseases, including allergy. Their effector functions are closely linked to their cytotoxic granule proteins. The release of the latter takes place by several different mechanisms, one of which is cytolysis, leading to the release of intact granules, so-called clusters of free eosinophil granules. The mechanism underlying this activation-induced form of cell death in eosinophils has remained unclear.

Methods: Isolated blood eosinophils were incubated on glass cover slips coated with immunoglobulins and complement 3c. A morphological characterization of the distinct stages of the proposed cascade was addressed by means of electron microscopy and immunohistochemistry. Experiments with pharmacological inhibitors were performed to underline the sequence of events within the cascade.

Results: Following eosinophil adhesion, we observed granule fusion processes, reactive oxygen species (ROS) production and early degranulation, leading to a distinct morphology characterized by cytosolic vacuolization. This cascade of events involved amplification steps and lead to a form of cell death characterized by loss of granule, vacuole, plasma membrane, and nuclear membrane integrity. On a molecular level, we demonstrated a signalling cascade, involving the β2-integrin Mac1, Fcγ-receptor III, phosphatidylinositol 3-kinases, p38 mitogen-activated kinase and the receptor-interacting serine/threonine-protein kinases 1 and 3.

Conclusion: We report that eosinophil cytolysis takes place by the adhesion-triggered RIP-1–RIP3-dependent form of cell death which is known as necroptosis.

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IgE antibodies, FceRIα and IgE-mediated local anaphylaxis can limit snake venom toxicity

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Background and objectives: Type 2 cytokine-related (i.e., type 2) immune responses associated with development of antigen-specific IgE antibodies can contribute to pathology in allergic diseases and to fatal anaphylaxis provoked by exposure of sensitized subjects to allergens, including those in foods, medicines, and animal venoms. However, our findings in mice indicate that IgE and its high affinity receptor, FceRI, also can enhance host defense against the morbidity and mortality induced by honeybee venom (BV) (Marichal T*, Starkl P* (* co-first authors), Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, Metz M**, Galli SJ** (** co-corresponding authors). *Immunity* 2013; **39:**963-75). In this study, we tested whether IgE antibodies, IgE-dependent effector mechanisms, and a local anaphylactic reaction to an unrelated antigen can enhance defense against Russell's viper venom (RVV) and determined whether such responses can be influenced by immunization protocol or mouse strain.

Methods: We compared the resistance of RVV-immunized wild-type, IgE-deficient, and *Fcer1a*-deficient mice following injection of a potentially lethal dose of RVV.

Results: A single prior exposure to RVV enhanced the ability of wild-type mice, but not mice lacking IgE or functional FceRI, to survive challenge with a potentially lethal amount of RVV. Moreover, IgE-dependent local passive cutaneous anaphylaxis in response to challenge with an antigen not naturally present in RVV significantly enhanced resistance to the venom. Finally, we observed different effects on resistance to RVV or honeybee venoms in BALB/c *versus* C57BL/6 mice which had received a second "immunization" exposure to that venom prior to challenge with a high dose of that venom.

Conclusions: These observations illustrate the potential benefit of IgE-dependent effector mechanisms in acquired host defense against venoms in mice, and suggest that developing IgE antibodies to as few as a single component of a venom can enhance resistance to the mortality induced by the whole venom, which consists of a diverse mixture of toxins. The extent to which type 2 immune responses against honeybee or Russell's viper venoms can decrease pathology associated with envenomation seems to be influenced by the type of venom, the frequency of venom exposure, and the genetic background of the host.

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Mast cells actively participate in tumor-promoting inflammation

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A hallmark of tumors is the infiltration of immune cells, which mirrors inflammatory conditions. Tumor-associated inflammation has been shown to contribute to tumor growth, by supplying for example proangiogenic factors that facilitate angiogenesis, invasion and metastasis. Mast cells have been found to actively participate in tumor-associated inflammation. In accordance, mast cell infiltrates have been correlated with tumor progression, prognosis and microvessel density in various neoplastic diseases.

To further elucidate the role of mast cells in tumor angiogenesis, we generated transgenic mice lacking mast cell-derived VEGF by crossing $VEGF^{fl/fl}$ mice to the Mcpt5Cre strain, which expresses Cre recombinase specifically in connective tissue type mast cells. Tumor growth of subcutaneously injected Lewis lung carcinoma cells (LLC) was significantly decreased in Mcpt5Cre/$VEGF^{fl/fl}$ mice as well as in mast cell-deficient Mcpt5Cre/iDTR mice. Moreover, induced depletion of mast cells in Mcpt5Cre/iDTR mice during tumor growth resulted in smaller LLC tumors. Of note, tumor growth was also reduced by treating growing tumors with the anti-VEGF antibody bevacizumab. To explore the interaction between mast cells and tumor cells in more detail, LLC cells were incubated with mast cell supernatant. We observed enhanced proliferation of LLC cells upon stimulation with mast cell supernatant. The anti-VEGF antibody bevacizumab inhibited mast cell-mediated proliferation of LLC cells. We next investigated mast cells in different histological subtypes of human lung adenocarcinoma by immunohistochemistry and found significantly increased numbers of mast cells. Patients with metastatic lung adenocarcinoma showed higher mast cell counts than non-metastatic patients. Furthermore, increased numbers of extensively degranulated mast cells were associated with metastatic patients, suggesting also enhanced activation of mast cells in metastatic lung adenocarcinoma.

Taken together, our findings demonstrate that mast cells promote growth of murine LLC tumors and the metastatic potential of human lung adenocarcinoma. Mast cell-derived VEGF may account for part of this effect. Hence, anti-VEGF antibodies may be a promising therapeutic approach in mast cell-associated tumors.

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Sputum cytokine signature in patients with severe asthma and local autoimmunity

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Background: With a repertoire of pro-inflammatory mediators, frequent exposure to degranulating immune cells and tissue injury, an 'inflamed' asthmatic lung potentially host conditions that could lead to loss of immune tolerance and generation of autoantibodies. Since different cytokine patterns are distinctive of the underlying inflammatory mechanism, we hypothesized that the cytokine/chemokine signature in sputum will identify patients with plausible 'autoimmune' type asthma.

Methods: Antibodies against eosinophil peroxidase (EPX) in the immunoprecipitated immunoglobulin (Ig) fraction of sputum supernatants were examined using an in-house ELISA. Additionally, a Line Immunoassay was used to screen anti-nuclear antibodies (ANAs) in the same samples. Finally, a multiplex platform was used to detect 65 cytokines /chemokines in the sputum supernatants of 62 patients with eosinophilic, neutrophilic or a pleotropic bronchitis.

Results: 22 out of 65 mediators documented significant variation in detection levels ($p < 0.01$) when compared to 14 healthy volunteers. 14 dominant variables allowed auto-formation of 2 distinct clusters using a 2-Step Cluster Analysis. 37.1% of the tested population comprising Cluster-2 (n=23) had increased levels of Interleukin (IL)-13, IL-5, eotaxin-2, chemokine (C-C) ligand 17 (CCL17) compared to Cluster-1 ($p<0.0001$), while the latter had increased levels of IL17A ($p=0.01$) and IL-1β ($p=0.05$). Furthermore, Cluster-2 charted increased presence of anti-EPX IgGs (1.09±0.646 vs. 0.68±0.63, $p=0.0039$) and ANAs (0.16±0.08 vs. 0.07±0.06, $p=0.0001$). Though ANAs significantly correlated with anti-EPX IgGs ($r=0.5131$, $p=0.0001$), a multivariate linear regression analysis illustrated difference in mediator predictability. Eotaxin-2, CCL21, IL-13 and CCL17 together best predicted the incidence of ANAs (adjusted $R2=0.215$, $p=0.004$); while IL-13, B-cell attracting chemokine (BCA-1), IL-23, fibroblast growth-factor 2 (FGF-2), together predicted anti-EPX IgGs (adjusted $R2=0.259$, $p<0.0001$). Both clusters had comparable sputum cell counts, lung function, and daily corticosteroid use. But, cluster-2 showed significant increase in airway degranulation as determined by free eosinophil granules (FEG) ($p=0.0004$) and EPX ($p=0.01$), which further correlated with ANA levels within the entire cohort (FEG: $r=0.4$, $p=0.004$; EPX: $r=0.5$, $p<0.0001$).

Conclusion: We hereby report a previously unrecognised subset of patients with asthma and luminal eosinophil degranulation who present with detectable levels of autoantibodies in sputum against autologous cellular components and a unique Th2 cytokine signature.

Funded by the Canada Research Chair Program and the Ontario Thoracic Society

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The Role of Mir-328 in Allergic Airway Disease

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Background: MicroRNAs (miRNAs) are small non-coding RNA that can bind to multiple target mRNA to repress protein production. Deregulation of miRNAs have been linked with pathogenesis of multiple human diseases. In immune cells, miRNAs regulate cell development, differentiation and production of inflammatory mediators. In our study, we have previously identified a novel role of miR-328 in regulating lung innate immunity during respiratory infection. Respiratory infection are often associated with exacerbation of airway diseases. Therefore, we aim to determine the functional roles of microRNA-328 (miR-328) in regulating allergic airway diseases.

Methods: Mice were sensitized with ovalbumin intraperitoneally and challenged with ovalbumin intranasally on day 13, 14, 15, and 16. Antagomir-328 were administered intranasally on day 13 and 15 to inhibit miR-328 expression in lungs. On day 17, airway resistance was measured using Buxco FinePointe resistance and compliance. Lung homogenates were used to isolate protein and RNA for cytokine analysis. To identify potential target for miR-328, we transfected biotin conjugated miR-328 mimic to macrophage cell line and subsequently pull down target mRNA using streptavidin magnetic beads. RNA samples were then analysed by gene microarray.

Results: Knockdown of miR-328 *in vivo* resulted in significant improvement in airway hyperresponsiveness, measured by reduction in airway resistance. Interestingly, we also observed similar improvement in airway hyperesponsiveness in an infection-induced steroid resistant asthma model. In addition, antagomir-328 administration also decreased IL-13 production and mucous secretion. Using RNA pull down with biotinylated miR-328 mimics, we has successfully identified 48 genes that are potetially targeted by miR-328.

Conclusion: Our study identify a novel role for miR-328 and provide proof of principle that miRNA pathways can be targeted in the lung and offer a potential new therapeutic approach for the treatment of allergic airway disease.

Grant Support: NHMRC project grant

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Global microRNA binding fine-tunes the transcriptome but profoundly alters the translatome: the application of RibomiR-seq to the understanding of epithelial cell activation in severe asthma

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Problem: The contribution of the transcriptome and proteome is not defined in severe asthma pathology. Transcription and translation correlate poorly, hence measures of total mRNA may misrepresent cell biology. More focused, polyribosome profiling and RNA-seq (Ribo-seq and Frac-seq) have revealed the complexity of translational regulation[1-3]. However, these approaches fail to incorporate information about microRNAs, key regulators of translation. **Method**: We have combined mRNA- and small- RNA-sequencing in total- and polyribosome- cellular fractions, which we have called RibomiR-seq. We applied RibomiR-seq to investigate primary bronchial epithelial cell activation in treatment-resistant severe asthma.

Results: We found both mRNA and microRNA differences in the polyribosome-bound fraction that were not apparent in total RNA. We show that there is genome-wide selective binding of mRNA isoforms to polyribosomes, which is dysregulated in asthma, and that microRNAs participate in this process. Moreover, microRNAs show different efficiency in regulating total and polyribosome-bound mRNAs: while they fine-tune total mRNA levels, they cause sharp changes in polyribosome loading of mRNAs. Dysregulated isoforms bound to polyribosomes map to pathways altered in severe asthma including inflammatory pathways distinct from the Th2-responses traditionally implicated in asthma, as well as pathways linked to cell metabolism, signaling and epithelial repair/remodeling. These pathways seem steroid insensitive, being dysregulated despite the presence of molecular markers of steroid delivery. This insight was not evident analyzing total aggregate genes and isoforms, indicating that assessment based solely on total mRNA changes fails to reveal fundamental features of cell activation.

Conclusion: Our results provide novel molecular insight into treatment-resistant severe asthma and mechanistic insight into microRNA regulation, demonstrating the fundamental importance of assessing polyribosomal-bound RNA populations (both mRNA and microRNA) to investigate genome-wide mRNA regulation.

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Comprehensive evaluation of serum periostin as a phenotype-specific biomarker in asthma

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Background: Periostin may be a novel phenotype-specific biomarker for Th2-driven asthma as suggested by its overexpression in epithelial cells from asthmatics, and upregulation by IL-4 and IL-13. However, clinical studies examining relationships between circulating periostin and airway eosinophilia are few and show conflicting results. Therefore, the suitability of periostin as a biomarker in asthma remains unclear.

Aim: To examine circulating periostin levels in well-characterised patients with asthma and thereby extend our knowledge regarding its levels in children and adults with different severities and treatment profiles. Furthermore, to examine the effects of corticosteroid treatment, acute exacerbations, stability over time and relationships with other biomarkers and clinical characteristics.

Methods: Serum periostin levels were examined by ELISA using 2 rat anti-human periostin mAbs (clones SS18A and SS17B), in subjects from the following 3 previously-described multicenter studies:

1. Swedish GALEN including 605 asthmatics with/without chronic rhinosinusitis (CRS), 110 individuals with CRS only, and 226 controls, aged 17-76 (Ek *Allergy* 2013;68:1314).

2. BIOAIR including 81 adult patients with severe asthma, 61 with mild-to-moderate asthma and 51 with chronic obstructive airway disease (COPD) (James *AJRCCM* 2015; epub ahead of print).

3. Swedish Search including school-age children (n=96) with various manifestations of persistent asthma (Konradsen *Pediatr Allergy Immunol* 2015; doi:10.1111/pai.12457).

Results: We show that in asthmatic patients, periostin levels are affected by age, BMI, lung function and smoking status (multiple regression analyses, $p<0.05$). Periostin levels were reduced by oral corticosteroid therapy (median 82 *vs* 68 ng/ml, $p<0.0001$), relatively stable over time (CV 14%, up to 6 visits) and not affected by acute exacerbations (median 92 *vs* 86 ng/ml, $p>0.05$). We also confirm previously observed correlations with sputum and blood eosinophils and exhaled NO (correlation coefficients 0.44, 0.38 & 0.25 respectively, $p<0.05$). However, no between-group differences were found when comparing periostin levels in different disease groups or healthy controls, and periostin did not associate with markers of airway inflammation in children.

Conclusion: We confirm associations between circulating periostin levels with markers of Th2-type inflammation, as well as lung function, and identify novel factors of importance to the use of periostin as a phenotype-specific biomarker in adult asthma.

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Usefulness of very low dose oral food challenge and oral immunotherapy

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Introduction We aimed to evaluate if the results of a very low (VL) dose oral food challenge (OFC) and oral immunotherapy (OIT) contributes to better management of food allergy.

Subjects and Methods We retrospectively reviewed definitive allergic subjects with cow's milk (CM) or wheat allergy who underwent the VL dose OFCs (heated milk: 3ml and wheat noodle: Udon 2g). Subjects who passed the OFC were defined as VL tolerant, and subjects who failed were defined as VL reactive. In addition to VL OFC, we have recently introduced a VL dose OIT. The VL dose OIT aims at 1/32 heated HE, 3ml CM, 2g of wheat noodle, and 0.5g of peanut. OFCs using VL doses of causative foods were performed with 32 patients; 6 negative cases were excluded, resulting in 26 cases in the study.

Results Of the 83 CM allergy subjects (median age, 4.3 y) who were included, 41 (49.4%) were VL tolerant, and 42 (51.6%) were VL reactive. Of the 57 included wheat allergy subjects (median age, 2.9 y), 32 (56%) were VL tolerant and 25 (44%) were VL reactive. VL OFCs allow the management of some low dose reactive CM or wheat allergic children to change from complete avoidance to partial intake of CM or wheat with better prognosis within a year. The subjects with OIT median milk-specific, egg white-specific, wheat-specific, and peanut-specific IgE levels were 39.4 kU/L, 44.1 kU/L, 151 kU/L, and 56.0 kU/L, respectively. The proportions of the subjects who were tolerant to VL of the causative foods after 1 year were 60% (6/10) for milk, 83% (5/6) for egg, 80% (4/5) for wheat, and 100% (5/5) for peanut. The adverse reactions by VL dose OIT was much less than those by a conventional OIT. In addition, immunological changes such as increase in Ag-specific IgG4 and decrease in Ag-specific IgE were observed.

Conclusion With food allergies, removing the need to eliminate a food that could be consumed in low doses could significantly improve quality of life and prognosis.

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Dual Variable Domain Antibodies, a novel platform for delivery of transformational efficacy to patients

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The dual variable domain immunoglobulin (DVD-IgTM) is a novel dual-specific IgG that simultaneously binds two mediators of disease by a single pharmaceutical entity. The molecule contains Fc and variable regions in a configuration similar to a conventional IgG; however, the DVD-Ig protein is unique in that each arm of the molecule contains two variable domains (VDs). Abbvie's lead DVD molecule, ABT-122 is an anti-TNF/IL-17 DVD-Ig capable of simultaneously binding and neutralizing both TNF and IL-17A. Phase I studies have shown acceptable safety, tolerability and pharmacokinetic profiles of ABT-122 that have enabled progression to Phase II studies in Rheumatoid Arthritis (RA) and Psoriatic Arthritis (PsA). In an effort to understand the mechanism of action, translation, and potential biomarkers for ABT-122 in clinical studies, both preclinical and clinical analyses were performed. Dual neutralization of TNF and IL-17 showed not only increased efficacy in a preclinical arthritis model compared to neutralization of either alone, but gene array analysis also identified chemokine pathways significantly affected by the combination treatment. Early clinical studies confirmed that ABT-122 modulates levels of certain serum chemokines, as well as expression of chemokine receptors, CXCR4 and CXCR5, on leukocyte subsets. In addition, we found increased IL-10 and decreased GM-CSF levels in LPS-stimulated PBMCs ex vivo from subjects receiving ABT-122. As these chemokine and cytokine pathways have been suggested to play a role in disease pathology or its resolution, these data indicate that dual blockade of TNF and IL-17 by ABT-122 could provide a unique new therapeutic approach for patients with RA and exemplifies the power of the DVD bispecific platform as a novel class of therapeutics for all immune mediated inflammatory diseases

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Blood eosinophils predict therapeutic effects of a GATA3 specific DNAzyme on both allergic early and late phase reactions in patients with asthma

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Backround: Th2 driven inflammation in asthmatic patients is controlled by GATA3 which is targeted by a novel GATA3 mRNA-specific DNAzyme (SB010). Recently we showed in mild allergic asthmatic patients that inhalation of SB010 significantly improved both allergic early (EAR) and late phase responses (LAR). Since blood eosinophil levels may serve as a marker to predict treatment responses, we evaluated SB010 treatment responses accordingly to blood eosinophil levels.

Methods: A secondary analysis based on pre-specified levels of relative blood eosinophil counts was performed in patients from a recent randomized, double-blind, placebo-controlled, multicenter clinical trial (Krug N et al. Allergen-induced asthmatic responses modified by a GATA3-Specific DNAzyme. NEJM 2015; 372:1987-1995; NCT: 01743768).

Results: The effect of SB010 treatment on both EAR and LAR responses continuously increased with higher relative levels of blood eosinophils. EAR responses improved by 39.8 % (blood eosinophils ≥ 3 %, p = 0.01), 46.3 % (blood eosinophils ≥ 4 %, p = 0.02) and 78.5 % (blood eosinophils ≥ 5 %, p = 0.02), respectively. Improvement of LAR responses ranged from 42.5 % (p = 0.01; blood eosinophils ≥ 3 %) to 48.9 % (p=0.05; blood eosinophils ≥ 4 %) and 49.4 % (p = 0.17; blood eosinophils ≥ 5%). Changes were accompanied by lower sputum levels of eosinophils and tryptase as well as by lower concentrations of blood IL-5 concentrations and exhaled NO.

Conclusion: Blood eosinophil counts may represent a suitable biomarker to stratify asthma patients for inhaled SB010 treatment.

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CompEx – a novel surrogate asthma exacerbation endpoint

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Background: Severe asthma exacerbations (SevEx) is the most important endpoint in clinical drug development, but due to the low event rate, exacerbation trials are traditionally not performed before phase 3 in development program.

Aims and Objectives: Establish a powerful composite endpoint of diary variables capturing clinically relevant deteriorations, combined with SevEx which allows phase 2 design of trials that are shorter in duration and involve fewer patients.

Methods: Data from seven 6–12 month trials (>14,000 patients) with budesonide/formoterol (Symbicort SMART®) using standardized collection of asthma exacerbations and diary card variables were investigated.

Diary events was created from available diary variables, i.e. PEF, reliever use, asthma symptoms and night-time awakenings. Predefined threshold values of deteriorations from baseline, and slopes to assess rate and direction of change identified diary events. Random Forest methodology identified the most predictive variables. A composite Exacerbation (CompEx) was defined as the first event of either a diary event or SevEx, and was evaluated using a Cox proportional hazards model. The performance of diary events and CompEx was assessed by comparing treatment effects as hazard ratios relative to SevEx.

Results: A CompEx algorithm based on morning/evening PEF, rescue SABA and asthma symptoms was associated with best overall performance and statistical power. When censored at 3 months, ~20% of patients experienced CompEx events vs. ~8% for SevEx, with small variations between studies. Treatment effect on CompEx at 3 months was well preserved, and reflected that on SevEx. The increased number of events in combination with the sustained effect resulted in a net gain in power allowing for a >50% reduction of patients needed. CompEx showed a similar effect profile as SevEx over time (-12 months), and in different subgroups of the patients.

Conclusion: CompEx is a surrogate endpoint that allows evaluation of exacerbation risk reduction in 3-month trials involving fewer patients compared with SevEx. Sponsor: AstraZeneca

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Probiotics attenuate mite allergen-induced allergic inflammation via PPARγ in the murine model of allergic diseases

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Abstract: Probiotics are normal inhabitants in the gastrointestinal tracts of man and are widely considered to exert a number of beneficial roles including immunomodulation, interference with enteric pathogens, and maintenance of a healthy intestinal microflora. In recent years, studies of probiotics have also confirmed their extra-intestinal effects, particularly for the prevention of allergic diseases. However, the anti-allergy mechanism of probiotics is still unclear. In this study, we found that continuous feeding of *Lactobacillus gasseri* (*L. gasseri*) 10^7 CFU/200 ml or 10^9 CFU/200 ml for 4 weeks in Der p-sensitized and challenged mice could prevent allergen-induced airway hypersensitivity and inflammation. There were also significant changes of T_H1 and T_H2 cytokine patterns, lymphocyte proliferations and immunoglobulin production between *L. gasseri*-treated and non-treated mice. Next, we applied microarray analysis of the lung draining lymph nodes and mesenteric lymph node of mice to detect genes expression signal pathways and genetic profiling of immunological tolerance induced by *L. gasseri* that plays an essential role of in the prevention and therapeutic effects on allergic asthma. We found that there was significantly decrease of inflammatory and chemokines genes expression and increased of carbohydrate and lipid metabolism genes expression in the *L. gasseri*-treated mice as compared to non-treated sensitized and challenged mice. One candidate targeted gene, PPARγ (peroxisome proliferator-activated receptor γ), have increased more than 10 folds after probiotic administration. We hypothesized that PPARγ may play an important protective role in allergen-induced airway inflammation. We have shown HDM-sensitized PPAR-γ P456L mutant mice have increased AHR to methacholine, more airway inflammatory cells infiltration and inflammatory cytokines secretion in bronchoalveolar fluid (BALF) as compared to those of wide type mice after HDM allergen provocation. Most significantly, probiotic administration could not reverse HDM induced airway inflammation in HDM-sensitized and challenged PPAR-γ P456L mutant mice. In summary, our results showed that PPAR-γ play important role in the inhibitory effect of allergen-induced airway inflammation in mice. And the anti-allergic effect on *L. gasseri* may through activation of PPARγ to alleviate airway inflammation in allergen-sensitized murine model of asthma.

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Direct Modulation of Dendritic and Epithelial Cell Responses by Human Milk Oligosaccharides.

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Introduction: We have previously demonstrated that Human Milk Oligosaccharides (HMOs) can attenuate symptoms in a mouse model of food allergy. It is currently unclear whether the beneficial effect of oral treatment with HMOs in allergy occurs through their prebiotic action or through direct modulation of immune responses. Here we test the effect of HMOs, 6'sialyllactose (6'SL) and 2'fucosylactose (2'FL), on dendritic cells (DC) and epithelial cell (EC) functions related to allergy.

Methods: The effects of HMOs on bone-marrow derived DC and T84 epithelial cell lines were studied, *in vitro*. The expression of maturation markers and cytokine release were assessed to measure the effect of HMOs on DC phenotype and function. Cytokine response of EC to TNF and antigen-antibody complex stimulation in the presence of HMO was also measured. Pharmacological inhibitors of cell signalling pathways were used to explore mechanism of action.

Results: 6'SL (1 mg/ml) but not 2'FL increased the population of DC expressing IL-10 (17.21 ± 1.62% vs 26.40 ± 2.07%, p= 0.0012) and the immunoregulatory enzyme, heme-oxygenase-1 (HO-1) (20.08 ± 2.141% vs 30.07 ± 3.336%, p= 0.0149). 6'SL also increased CpG- and LPS- induced IL-10 release while decreasing IL-12, suggesting the induction of a tolerogenic type of DC. Use of a PPARγ antagonist resulted in attenuation of the 6'SL-induced changes in DC.

With regard to EC function, 6'SL, but not 2'FL, exposure resulted in an inhibition of TNFα and antigen-antibody complex induced IL-8 release from T84 cells. As in DC, a PPARγ antagonist blocked the inhibitory effect of 6'SL on IL-8 production by EC. Both 6'SL and 2'FL abolished antigen-antibody complex induced release of the chemokine CCL20, but 2'FL did so at a 1000 fold lower concentration than 6'SL (10 μg/ml vs 10 mg/ml) and correspondingly reduced NFκB activity at these concentrations. Unlike the effects on IL-8 release, inhibition of CCL20 and NFκB activity were independent of PPARγ.

Conclusion: The HMOs 6'SL and 2'FL can directly modulate immune cell responses related to allergy, utilizing distinct PPARγ dependent and independent pathways respectively. Such effects may contribute to the ability of HMOs to reduce allergy symptoms.

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Protection from food allergy development is associated with distinct characteristics in an oral mouse immunization protocol

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Background: We have previously established a murine food allergy model based on allergen feedings under gastric acid-suppression, which is associated with elevated allergen-specific IgE levels, anaphylaxis and elevated Th2 cytokines. However, not all animals treated according to this protocol develop elevated allergen-specific IgE upon oral sensitization. Therefore, in this study we aimed to determine characteristics of animals being protected from food allergy as our group of interest and compared them to animals with elevated levels of specific IgE and IgG1 as well as anaphylactic responses.

Methods and Results: Out of the 64 BALB/c animals being subjected to our oral ovalbumin (OVA) immunization protocol under gastric acid-suppression, 10 animals (16%) did not show any elevation of OVA-specific IgE or IgG1 titers indicating protection from allergic sensitization. For comparison, 10 mice developing highest OVA-specific IgE and elevated IgG1 titers were chosen. Allergen challenges confirmed reduced antigen uptake and lack of clinical symptoms in our group of interest, while in the allergic controls anaphylaxis could be objectified by high levels of mouse mast cell protease-1 (mMCP-1) and a drop of core body temperature. While response to acid suppression medication was comparable between the two groups, significantly lower numbers of CD4+ T cells and regulatory T cells were detected in the non-responders. In animals protected from food allergy development significant lower levels of IL-4, IL-5, IL-10 and IL-13 were measured in supernatants obtained from stimulated spleen cells. Only for IL-22 comparable levels were found between the two groups. These findings were accompanied by significantly increased numbers of total lymphocytes and reduced numbers of monocytes, erythrocytes and hematocrit in the peripheral blood of the non-responders. Interestingly, comparison of microbiota revealed differences regarding the composition of bacterial communities on single bacterial Operational Taxonomic Unit (OTU) level between protected and allergic mice.

Conclusions: These data clearly indicate that protection from food allergy development in our mouse model was associated with specific pattern of cytokine levels, blood cell count as well as significant differences of single bacterial OTUs resulting in distinct microbiota composition.

Supported by FWF grants P21884, P21577 and KLI284 of the Austrian science fund FWF. HF is supported by the Deutsche Forschungsgemeinschaft (Cluster of Excellence 'Inflammation at Interfaces' EXC 306).

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Skin barrier homeostasis and its failure in atopic disorders

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Enhanced cutaneous sensitization by skin barrier dysfunction has been extensively investigated as an initial important step for the onset of allergic disorders. When activated, Langerhans cells (LCs) extend their dendrites above tight junction (TJ), capture external antigens which penetrated through stratum corner (SC), and migrate to lymph nodes. SC is consist of stacked dead corneocytes which are generated from terminally-differentiated keratinocytes in stratum granulosum (SG). SG has at least three layers; SG1 to SG3 from top to bottom. TJ is always formed between SG2 cells, leaving SG1 cells out as a thin layer of liquid phase. The purpose of this study is to isolate and characterize SG1 cells, which show live-to-dead transition of keratinocytes.

To perform single cell analysis of SG1 cells, we generated EGFP-knock-in mice in the SASPase gene locus, which contains SG1-specific promoter. SASP(EGFP) mice showed SG1-specific expression of GFP in the epidermis. By optimizing the trypsin treatment of the epidermal sheet, we could identify GFP-positive SG1 cells and GFP-negative SG2 cells. Confocal microscopic analysis revealed that these isolated SG cells have polygonal domed saucer-like morphology, suggesting the maintenance of their unique in vivo cell shape and structure. We examined various culture conditions including [Ca2+] and pH. High [Ca2+] under weakly acidic pH induced cell death of most SG cells accompanied by gradual degradation of granules, whereas low [Ca2+] under neutral pH did not. In vitro Ca2+ imaging by Rhod 2-AM revealed a transient intracellular [Ca2+] increase preceding the granule degradation. These results suggest that the initiation

of cornification process is regulated by the extracellular environment of SG1 cells including [Ca2+] and pH. Furthermore, this novel culture system will provide a useful tool to understand the detailed molecular mechanisms for cornification and SC barrier formation.

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Inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5) affects epidermal morphology in constitutive knockout mice and could be a novel key player in delayed type hypersensitivity responses of the skin

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Inter-alpha-trypsin inhibitors (ITIs) are protease inhibitors that are thought to be important regulators in various acute-phase processes. They are composed of one light chain (bikunin) and different heavy chains (ITIHs). The only function known so far of ITIHs is the covalent linkage to hyaluronic acid (HA). Using GeneChip® Human Exon 1.0 ST expression profiling we identified ITIH5 as the major ITIH family member expressed in human skin. To investigate the role of ITIH5 in skin we established a new ITIH5$^{-/-}$ mouse model. We detected that the skin of ITIH5$^{-/-}$ mice as well as corresponding *in vitro* 3D-skin-equivalents exhibited structural abnormalities. In both models a significantly reduced epidermal thickness and absence of a stratified structure as well as a complete lack of the *stratum corneum* was observed. Interestingly, using a Van-Gieson staining we detected different extracellular matrix (ECM) structures in skin-equivalents of ITIH5$^{-/-}$ and wild type mice. First results indicate a mechanistic link between the ability of ITIH5 to stabilize the ECM-component HA and the impaired ECM structure if ITIH5 is lacking. Moreover, ITIH5 expression is significantly up-regulated in various inflammatory skin diseases including allergic contact dermatitis (ACD). To understand more precisely the role of ITIH5 in ACD we used the contact hypersensitivity (CHS) mouse model, where a role for HA degradation in modulating the inflammatory response has been recently described. Preliminary studies revealed that ITIH5$^{-/-}$ mice showed significantly reduced CHS responses. In consideration of these observations we assume that ITIH5 could be a novel key player in delayed type hypersensitivity (DTH) responses of the skin.

Taken together, our experiments revealed to our knowledge for the first time the specific and strong expression of ITIH5 in human skin. Preliminary evidence indicates that ITIH5 forms complexes with HA, thereby on the one hand facilitating the formation of a normal ECM structure and on the other hand modulating CHS responses.

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Epicutaneous allergic sensitization by synergy between allergen protease-activity and mechanical skin barrier damage in mice

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Allergen sources such as mites, insects, fungi, and pollen contain proteases. Airway exposure to protease allergens induces allergic airway inflammation and IgE/IgG1 responses via IL-33-dependent mechanisms in mice [1]. However, in spite of recent advances in our understanding of the roles of protease allergens upon airway exposure, the involvement of these allergens in epicutaneous (e.c.) allergic sensitization remains largely unknown. We recently reported a novel murine model of e.c. sensitization to papain via intact skin, in which specific IgE/IgG1 production was induced in a manner dependent on the protease activity of papain [2].

We herein examined the e.c. sensitization of mice to a model protease allergen, papain, the effects of tape-stripping, which induces epidermal barrier dysfunction, and the atopic march upon a subsequent airway challenge. Papain painting on ear skin and tape-stripping synergistically promoted dermatitis in ear skin, the upregulation of serum total IgE, and induction of papain-specific IgE/IgG1. E.c. sensitization induced papain-specific Th2 and Th17 differentiation in draining lymph nodes. Ovalbumin and protease inhibitor-treated papain induced no or weak responses, while the co-administration of ovalbumin and papain promoted induction of ovalbumin-specific IgE/IgG1. Wild-type and IL-33-deficient mice showed similar responses in the e.c. sensitization phase. The subsequent airway papain challenge induced airway eosinophilia and maintained high papain-specific IgE levels in wild-type, but not IL-33-deficient mice.

These results suggest that allergen source-derived protease activity and mechanical barrier damage such as that caused by scratching synergistically promote e.c. sensitization and skin inflammation, and that IL-33 is dispensable for e.c. sensitization, but is crucial in the atopic march upon a subsequent airway low-dose encounter with protease allergens.

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Human rhinovirus infected epithelial cells produce chemoattractants for fibroblasts.

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Background: Thickening of the lamina reticularis is a characteristic feature of remodeling in the asthmatic airways. This thickening is now known to be present in young children who wheeze. Human rhinovirus (HRV) infection is a common trigger for childhood wheezing, and is a risk factor for subsequent asthma development. Children can have multiple HRV infections each year. We hypothesized that HRV-infected epithelial cells release chemoattractants to recruit fibroblasts towards the epithelium. Subepithelial fibroblasts could contribute to further thickening of the lamina reticularis. We, therefore, investigated whether conditioned medium from HRV-infected epithelial cells can trigger directed migration of fibroblasts.

Methods: Human bronchial epithelial cells and fibroblasts were isolated from normal lungs not used for transplant**.** Human bronchial epithelial cells were exposed to medium alone or infected with HRV-16. Conditioned medium from both conditions were tested as chemoattractants for human bronchial fibroblasts from multiple donors in both a Boyden chamber and in the xCELLigence cell migration apparatus. Platelet derived growth factor (PDGF) was used as a positive control stimulus.

Results: HRV conditioned epithelial medium was chemotactic for fibroblasts in both the Boyden chamber and the xCELLigence system. Migration was found to peak at 6 h. Production of epithelial chemoattractants required HRV replication. Treatment of fibroblasts with pertussis toxin, an inhibitor of Gai-coupled receptors, prevented their migration to HRV conditioned epithelial medium but not to PDGF. Multiplex analysis of epithelial supernatants identified CXCL10, CXCL8 and CCL5 as Gai-coupled receptor agonists of potential interest. Subsequent analysis confirmed that fibroblasts express CXCR3 and CXCR1, but not CXCR2 receptors. Inhibition studies with neutralizing antibodies and receptor antagonists established that CXCL10 and, to a lesser extent, CXCL8, but not CCL5, contribute to fibroblast migration caused by HRV conditioned epithelial medium.

Conclusion: CXCL10 and CXCL8 produced from HRV-infected airway epithelial cells are chemotactic for fibroblasts. This raises the possibility that repeated HRV infections in childhood could contribute to the initiation and progression of airway remodeling that is a characteristic feature of asthma by recruiting fibroblasts that produce matrix proteins leading to thickening of the lamina reticularis.

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Vertical Transmission of Respiratory Syncytial Virus (RSV) to Fetuses *In Utero* Alters Post-Natal Th1 & Th2 Cytokine Levels in Weanling Rats Re-challenged with RSV

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Rationale: Respiratory syncytial virus (RSV) is strongly associated with the development of asthma during childhood. As previous studies in our laboratory have shown post-natal changes in airway reactivity upon re-challenge RSV infection in weanling rats exposed to RSV *in utero*, we hypothesized that prenatal RSV exposure could result in lowered Th1 cytokine levels and a skewing towards a Th2-type anti-viral response.

Specific Aim: To measure the post-natal serum cytokine levels of *in utero* RSV-exposed weanling rats re-challenged with either single (+2-weeks) or sequential (+2 and +4-weeks of age) RSV infections.

Methods: On Day 12 of pregnancy, dams received intratracheal instillations of sterile media (C) or RFP-tagged RSV (R). At +10 days of age, pups from each dam treatment group received intratracheal instillations of sterile media (CC or RC) or RFP-tagged RSV (CR or RR). On Day +15, five days post-infection, a population of subjects in each group had serum harvested from whole blood. For remaining subjects in each treatment group, the inoculation regimen was either then repeated at +23 days of age or bypassed. Five days after the second re-challenge RSV infection (+28 days of age), serum was isolated from whole blood.

Results: Weanling rats exposed to RSV *in utero* who received a single RSV re-challenge infection (RR) displayed lower levels of the Th1 cytokines IL-2 and IL-18 and pro-inflammatory cytokine IL-17A at +2-weeks of age compared to those lacking *in utero* RSV exposure (CR). These altered Th1 cytokine trends remained at +4-weeks of age upon secondary RSV re-challenge infection but had dissipated if secondary RSV re-challenge did not occur.

Conclusions: Exposure to RSV *in utero* conveys imbalances in Th1 and Th2 immune cytokine and chemokine levels upon post-natal RSV re-challenge infection in weanling rats. Levels of the specific Th1 cytokines IL-2 and IL-18 remain suppressed with additional RSV re-challenge infections, suggesting *in utero* RSV exposure may impart a self-tolerance phenotype allowing future RSV infections to be more severe and cause heightened airway reactivity due to poor infection defense and clearance.

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Interleukin-13 induces glucocorticoid-insensitive hyperreactivity of human small airways

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This study aimed to investigate whether IL-13, a key mediator in the pathobiology of asthma, can directly alter human airway smooth muscle (HASM) function in isolated human small bronchi. Human small bronchi (diameter <2 mm), isolated from human lung tissue obtained after lobectomy, were cultured for two days with or without IL-13 (100 ng/ml). Subsequently, contractile and relaxant responses of human bronchi were studied using myographs. The effect of IL-13 on histamine-induced mobilization of intracellular Ca^{2+} was visualized with Fluo-4 in HASM cells. In human small bronchi, IL-13 induced a 2.5-3.1 fold increase in the potency of contractions towards histamine and carbachol, whereas no effects on the maximal amplitude of these contractions were observed. On histamine pre-contracted bronchi, IL-13 reduced the efficacy and potency for the relaxation to salbutamol, whereas relaxations induced by formoterol and the TAS2R agonists chloroquine and noscapine remained unaltered. Intracellular Ca^{2+} measurements in HASM cells revealed that IL-13 increased both the potency and maximal amplitude of histamine-induced Ca^{2+} mobilization. Dexamethasone (100 nM) did not prevent the IL-13-induced hyperreactivity in either human bronchi or HASM cells.
This study demonstrates that IL-13 induces glucocorticoid-insensitive, functional remodeling of human airway smooth muscle that can contribute to the development and persistence of airway hyperresponsiveness by a direct action on the airway smooth muscle.

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Pollen Extracts and Cat Dander Extract Require Myeloid Differentiation Factor 2 (MD2) and MyD88 adaptor to stimulate innate neutrophil-mediated allergic sensitization

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Background. Neutrophils have long been viewed as terminally differentiated cells that clear extracellular pathogens like bacterium. However, a growing body of literature indicates that neutrophils have numerous additional effects that regulate innate and adaptive immune responses. We have recently reported that intrapulmonary challenge of mice with ragweed pollen extract (RWPE) stimulates a TLR4-dependent neutrophil recruitment to the lungs. Adoptive transfer of neutrophils to the lungs of mice that lack TLR4 and cannot be sensitized to RWPE reconstitutes the ability of repeated RWPE challenges to stimulated allergic sensitization, indicating that RWPE-induced innate neutrophil recruitment stimulates allergic sensitization to RWPE. Here we sought to determine the role of Myeloid Differentiation Factor 2 (MD2), a TLR4 co-receptor, and MyD88 and TRIF, the TLR4 adaptors, in RWPE, other pollen extracts, and Cat Dander (CDE) induced allergic sensitization and allergic airway inflammation.

Methods. CDE, RWPE and other pollen extracts containing very low levels of endotoxin (< 0.1pg LPS /1µg allergenic extract protein) were purchased from Greer Laboratories, NC and used in all experiments. HEK cells that expressed only TLR4 or cells that expressed both TLR4 and MD2 were stimulated with RWPE, CDE, other pollen extracts, and secretion of CXCL chemokines were quantified. Mice with siRNA knockdown of MD2 or mice lacking MyD88 or TRIF adaptors were intranasally challenged once with RWPE or CDE to quantify innate inflammation in the lungs, or 6 times to quantify allergic sensitization (defined as an increase in allergen-specific IgE in serum) and allergic lung inflammation.

Results. CDE, RWPE and many other pollen extracts stimulated CXCL8 secretions only in cells expressing both TLR4 and MD2, but not those expressing TLR4 alone. Knockdown of MD2 attenuated RWPE or CDE innate neutrophil recruitment, allergic sensitization and allergic airway inflammation. Disruption of TRIF increased, whereas disruption of MyD88 abrogated CDE and RWPE-induced innate neutrophil recruitment, allergic sensitization and allergic airway inflammation.

Conclusions. Pollen extracts and Cat Dander extract utilize a shared pathway consisting of MD2 receptor in the context of TLR4, and MyD88 as a shared TLR adaptor. Stimulation of this pathway induces an innate neutrophilic lung inflammation that in turn stimulates allergic sensitization and allergic lung inflammation. Molecules that target this signaling pathway are likely to prevent allergic sensitization to many environmental allergens.

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Water soluble chitosan inhibits nerve growth factor in murine model of mite-allergen induced allergic rhinitis

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Allergic rhinitis (AR) is characterized as nasal airway hyperresponsiveness and nasal mucosal inflammation disease mediated by IgE-associated processes. Growing information illustrates that nerve growth factor (NGF), a neurotrophin, plays an important role in bidirectional signaling between neuroimmune responses by augmenting an existing TH2 immune response and vice versus. Since water-soluble chitosan (WSC) have been demonstrated to have anti-inflammatory properties that could inhibit the development of allergic TH2 response, we aimed to assess the effect of WSC on the NGF in a mouse model of Dermatophagoides pteronyssinus (Der p)-induced allergic rhinitis. First of all, we established a NGF-mediated AR toward augmenting systemic total and Der p-specific IgE levels, upper airway hyperresponsiveness, and local immune-mediated inflammatory response of the infiltration of eosinophils and degranulation of mast cells as well as Th2 related cytokines production in the nasal septum and nasal cavity lavage fluids. Moreover, type 2 innate lymphoid cells (ICL2) were significantly increased in the cervical draining lymph nodes in AR mice. Interestingly, intranasal administration of WSC attenuated allergic inflammation and decreased AHR and lowered ILC2 cells infiltration in the treated mice. The expression of NGF and its high affinity tyrosine kinase receptor A (TrkA) receptors in nasal epithelium of Der p-stimulated mice were also lowered after WSC administration. Next, we used human nasal septum epithelial cell line (RPMI-2650) to investigate the detail mechanisms of candidate anti-allergic agents—WSC in attenuating Der p-induced airway inflammation. The results showed that NGF and TH2 related cytokines create an amplification loop resulting in broader allergic inflammation in upper-airway epitheliums. In addition, WSC attenuated allergic inflammation through inhibiting NGF biosynthesis and as well as its functions of in addition to directly epithelial damaging effect during the allergic TH2 immune responses. In summary, we have demonstrated the role of NGF in a mouse model of house dust mite-induced AR, and the therapeutic effect of water soluble chitosan (WSC) in our AR mouse model , may through the attenuation NGF-induced airway inflammation as well as the inhibition of NGF synthesis. Our results also provide a new therapeutic modality of patients suffered with AR in clinical condition.

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Insulin Regulates Proteinase-Activated Receptor-2 Expression on Airway Epithelium

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Introduction: Activation of Proteinase-Activated Receptor-2(PAR-2), a pro-inflammatory receptor for aeroallergens and endogenous serine proteinases, mediates allergic sensitization and allergic airway inflammation in animal models of asthma. PAR-2 expression is increased on the airway epithelium of asthmatic individuals compared to healthy controls, but the factors responsible for increased expression as well as the consequences of increased PAR-2 expression are unknown. We hypothesize that cellular stress, a characteristic of inflamed airways diseases, regulates PAR-2 expression on airway epithelium.

Methods: The growth factors we added to the airway epithelial cell culture media are bovine pituitary extract, epidermal growth factor and insulin. Normal human bronchial epithelial cells (NHBE) were cultured with or without growth factors (growth factor deprivation), or in the absence of individual growth factor for up to 48h. PAR-2 mRNA levels were studied by qRT- PCR. PAR-2 function was studied by measuring PAR-2-mediated calcium release from intracellular stores using a fluorescence-based assay.

Results: We have shown that growth factor deprivation, but not oxidative stress or hypoxia, upregulates PAR-2 mRNA and protein expression in NHBE cells. By excluding individual growth factors in our culture we now show that growth factor deprivation-induced PAR-2 upregulation was the result of exclusion of insulin (insulin deprivation). Insulin deprivation for 24h and 48h increased PAR-2 mRNA levels by 1.7+/-0.1 fold (n=9) and 2.3+/- 0.3 fold (n=4) respectively, compared to cells grown in the presence of growth factors. Addition of insulin reversed PAR-2 upregulation in insulin-deprived cells, but also in growth factor deprived cells. Insulin deprived cells showed increased intracellular calcium release upon PAR-2 activation compared to cells grown in the presence of insulin, indicating that the increased PAR-2 mRNA expression leads to increase PAR-2 function. Insulin deprivation also increased PAR-2 promoter activity, indicating that insulin deprivation-mediated upregulation in PAR-2 mRNA is due to increased PAR-2 gene transcription. Our preliminary results suggest that insulin deprivation- mediated PAR-2 upregulation is extracellular-signal-regulated kinase 1/2 (ERK1/2) dependent.

Conclusions: Insulin may regulate PAR-2 expression on airway epithelial cells and consequently regulate epithelium-mediated inflammation in the airways. Further understanding of PAR-2 regulation in airway epithelial cells may lead to novel treatments for inflammatory diseases such as asthma.

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Roles of C-type lectin receptors Dectin-1/2 in house dust mite-induced allergic fibroblasts

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Background: Asthma is a complex human disease characterized by allergic airway inflammation and airway hyperresponsiveness (AHR). The fact that sensitization against fungi is closely related to the severity of asthma suggests that immune systems recognizing fungi are involved in the pathogenesis of severe asthma. Recently, C-type lectin receptors, Dectin-1 (gene symbol *Clec7a*) and Dectin-2 (*Clec4n*), have been shown to function as not only major pattern recognition receptors for fungi but also receptors for some components of house dust mite (HDM) extract, a major allergen for asthma. However, the roles of Dectin-1 and Dectin-2 in the induction of HDM-induced allergic airway inflammation remain largely unknown.

Objectives: To determine the roles of Dectin-1 and Dectin-2 in HDM-induced allergic airway inflammation.

Methods: We examined the roles of Dectin-1 and Dectin-2 in the induction of HDM-induced Th2 and Th17 cell differentiation and subsequent allergic airway inflammation by using *Clec7a*-deficient (*Clec7a*$^{-/-}$) mice *and Clec4n*-deficient (*Clec4n*$^{-/-}$) mice. We also investigated Dectin-1- or Dectin-2-expressing cells in the lung and their roles in HDM-induced allergic airway inflammation.

Results: Both *Clec7a*$^{-/-}$ mice and *Clec4n*$^{-/-}$ mice showed significantly attenuated HDM-induced allergic airway inflammation and decreased Th2 and Th17 cell differentiation as compared with wild-type (WT) mice. Dectin-1 was expressed on CD11b^{+} dendritic cells (DCs) but not on CD4^{+} T cells or epithelial cells in the lung. Dectin-2 mRNA, together with FcRγ mRNA, was also expressed in CD11b^{+} DCs in the lung. CD11b^{+} DCs isolated from *Clec7a*$^{-/-}$ mice or *Clec4n*$^{-/-}$ mice expressed lower levels of proinflammatory cytokines and co-stimulatory molecules which could lead to Th2 and Th17 cell differentiation than those from WT mice.

Conclusion: Dectin-1 and Dectin-2 were expressed on CD11b^{+} DCs and promote HDM-induced Th2 and Th17 cell differentiation and allergic airway inflammation.

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Allergen-exposed bronchial epithelial cell-stimulated migration of smooth muscle is suppressed by inhaled corticosteroid and long acting beta-agonist therapy

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Background: Inhaled corticosteroids are the cornerstone of asthma treatment and although they are significantly effective in suppressing airway inflammation, the underlying airway remodeling is relatively resistant. There is evidence that airway epithelial cells, in response to allergen, secrete factors that induce myofibroblast development and migration toward the epithelium. Inhaled long-acting b_2-agonist (LABA) is added to corticosteroid therapy when patients remain symptomatic despite corticosteroid treatment, and this addition results in improved symptom control and a reduced risk of asthma exacerbations. However, whether combination therapy is able to impact airway remodeling remains controversial. We hypothesized that *allergen exposure triggers the release of factors from airway epithelium to stimulate migration of airway smooth muscle cells toward the epithelium and that this migration could be suppressed by treatment with corticosteroid + LABA*.

Methods: Primary cultures of human bronchial epithelial cells derived from normal subjects (non-transplanted lungs), and bronchial brushings from asthmatic subjects were exposed to house dust mite (HDM) extract or medium (control) alone, and the conditioned media collected after 24 h. Primary airway smooth muscle (ASM) cells obtained from normal subjects were exposed to HDM or control conditioned medium and the migratory response compared using the xCELLigence real time cell analyzer (Roche Applied Science). In separate experiments, the ASM cells were pre-treated with corticosteroid + LABA or vehicle.

Results: ASM cells have a greater migratory response to HDM conditioned medium compared to control medium. This response was amplified when HDM conditioned medium was obtained from bronchial epithelial cells from asthmatic compared to normal subjects and was inhibited by corticosteroid + LABA combination.

Conclusion: Our data provides evidence that HDM stimulates bronchial epithelial cells to secrete mediators that can promote the migration of ASM cells. Epithelial cells derived from asthmatic patients induce greater migration. Furthermore, the response to epithelial-derived mediators can be mitigated by pretreatment of ASM cells by corticosteroid + LABA combination therapy. These data may have implications regarding the pathogenesis of airway remodeling in asthma and for the treatment and prevention of airway remodeling.

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Immunoglobulin G to 91 allergenic molecules in early childhood by route of exposure, current and future allergic sensitization: a MAS birth cohort study

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Background: Studies of a limited number of allergenic sources have suggested that non-sensitized children produce immunoglobulin G (IgG) responses mainly to foodborne allergens, while IgE-sensitized children also produce strong IgG responses to the respective airborne molecules.

Objective: We sought to test with a comprehensive methodology the hypothesis that both the route of exposure and an allergic background affect the IgG responses to a broad array of allergenic molecules in early childhood.

Methods: We examined sera of 148 children participating in the German Multicentre Allergy Study, a birth cohort born in 1990. IgG to 91 molecules of 42 different sources were tested in a multiplex microarray (ISAC™, TFS, Sweden, cut-off 0.1 ISU/L). The allergenic molecules categorized by route of exposure (animal foodborne, vegetable foodborne and airborne) were studied at a molecular and source level. Allergic sensitization at age 2y and 7y was defined by IgE≥0.35 kU/L to ≥1 of 8 or 9 extracts from common allergenic sources, respectively.

Results: The prevalence and concentration of IgG to allergenic molecules in non-sensitized children at age 2y were extremely heterogeneous, headed by the category of animal food (87±13%; 61 ISU/L, CI 95% 52-71), intermediate for vegetable food (48±27%; 13 ISU/L, CI 95% 11-16) and lowest for airborne allergens (24±20%; 3 ISU/L, CI 95% 2.4-3.4) (p for trend <0.001 [%], p for trend <0.001 [levels]). Significantly higher frequencies for each category but in the same order (animal food>vegetable food>airborne) were observed in IgE-sensitized children at age 2y, when compared to their non-sensitized peers. Weaker associations could be observed between IgG responses at 2y of age and allergic sensitization at 7y of age.

Conclusion: The children's repertoire of IgG antibodies at 2 years of age to a broad array of animal foodborne, vegetable foodborne and airborne allergenic molecules is profoundly dependent on the route of allergen exposure and the child's allergic sensitization status.

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Activation of peroxidases causes airway inflammation via production of hypothiocyanite

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Background: We previously showed that pendrin/Slc26a4, an anion transporter, is a downstream molecule of IL-4/IL-13, and that it plays an important role in the pathogenesis of airway inflammation. Furthermore, we recently found that peroxidases accelerate airway inflammation. Thiocyanate (SCN^-) is an anion transported into pulmonary lumens via pendrin. Peroxidases catalyze the reaction of SCN^- together with hydrogen peroxide, which results in the production of hypothiocyanite ($OSCN^-$). $OSCN^-$ serves as a potent innate defense system against microbes in the lung. It is, however, still unknown whether $OSCN^-$ has any detrimental functions on epithelial cells leading to airway inflammation.

Methods: We established an *in vitro* $OSCN^-$ production system to investigate whether $OSCN^-$ caused inflammation or cell death by acting on airway epithelial cells. In this system, $OSCN^-$ was generated by oxidation of SCN^- using lactoperoxidase, and we cultured the airway epithelial cells under several concentrations of $OSCN^-$. We then studied the activation of NF-κB in response to $OSCN^-$ and its molecular mechanisms. In addition, we evaluated cell death in the presence of $OSCN^-$ by flow cytometry.

Results: $OSCN^-$ in low doses activated NF-κB in the airway epithelial cells. This $OSCN^-$-activated NF-κB consisted of at least p50. Protein kinase A (PKA), which is a sensor of oxidants, was dimerized in response to low-dose $OSCN^-$ and the dimerized PKA, the activated form, was required for the activation of NF-κB. Furthermore, $OSCN^-$ in high doses caused necrosis of airway epithelial cells, followed by the release of IL-33 from their nuclei.

Conclusions: We have shown here that $OSCN^-$ produced by peroxidases affects airway epithelial cells, thereby leading to possible airway inflammation. These results suggest that the peroxidase-$OSCN^-$ pathway may be a novel therapeutic target for airway inflammation including bronchial asthma.

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Targeting Nrf2 by triterpenoid CDDO-Me inhibits IL-33 secretion and improves Th2-type airway inflammation induced by natural airborne allergen exposure

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Background: Th2-type immune responses to environmental allergens may play a major role in pathophysiology of asthma. Oxidative Stress induced by reactive oxygen species (ROS) are involved in a variety of biological and immunological processes. The transcription factor Nrf2 activates a number of genes involved in host's antioxidant activities.

Objective: The goal of this project was to investigate the roles of oxidative stress in allergic immune responses and to examine the therapeutic effects of an Nfr2 activator triterpenoid CDDO-Me.

Methods: Normal human bronchial epithelial (NHBE) cells were exposed to extracts clinically-relevant fungus Alternaria and secretion of IL-33 was examined by ELISA. Naïve Balb/c mice were intranasally exposed to Alternaria extracts, and the expression of type 2 cytokines and antioxidant molecules were examined in BAL fluids and lung homogenates.

Results: NHBE cells produced ROS intracellularly and secreted IL-33 when they were exposed to Alternaria. An ROS scavenger glutathione (GSH) inhibited both ROS production and IL-33 release. Similarly, when mice were exposed to Alternaria, they secreted IL-33 into BAL fluids and produced IL-5 and IL-13 in the lungs, both of which were inhibited by airway administration of exogenous GSH. Treatment of mice with CDDO-Me enhanced expression of a number of endogenous antioxidant molecules, such as glutathione S-transferase and thioredoxin reductase 1. Furthermore, CDDO-Me significantly inhibited eosinophilic airway inflammation, mucus production and increased plasma levels of IgE in mice that are exposed to Alternaria for a prolonged period.

Conclusion: Oxidative stress responses in airway epithelial cells likely play important roles in IL-33 secretion and type 2 airway immune response when animals are exposed to airborne allergens. Increased protection against oxidative stress by treatment with new antioxidant agents, such as CDDO-Me, may be beneficial as a therapeutic strategy for allergic airway diseases and asthma.

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Leukotriene C_4 Induces Pulmonary Eosinophilia by an Interleukin 33-Driven Mechanism

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Interleukin 33 (IL-33) is a potent inducer of type 2 immune responses to viral infections, helminths, and environmental proteases. The mechanisms responsible for the production and release of IL-33 are incompletely understood. We previously demonstrated that IL-33 is highly expressed in the sinonasal tissue of patients with aspirin exacerbated respiratory disease (AERD), a clinical syndrome characterized by severe sinonasal and pulmonary eosinophilic inflammation and pathognomonic cysteinyl leukotriene (cysLT)-dependent reactions to nonselective cyclooxygenase (COX) inhibitors. We also demonstrated that cysLT-deficient mice were protected from upregulated expression of IL-33 in a mouse model of AERD, along with attendant pulmonary eosinophilia. To determine whether cysLTs could directly induce immunopathology through IL-33, we employed a model of inhalation challenge with LTC4, the parent cysLT, in wild-type C57BL/6 mice sensitized with ovalbumin (OVA) and challenged by inhalation with low-dose OVA. LTC_4 markedly potentiated airway eosinophilia, accompanied by significant increases in lung levels of ILC2s. These responses were blocked by depletion of platelets, and were blunted in mice bearing a targeted deletion of the type cysLT receptor ($CysLT_2R$). Antibody-mediated blockade of IL-33 or depletion of ILC2s suppressed the LTC_4-mediated induction of eosinophilia. These studies confirm that cysLTs can promote tissue production and release of IL-33 as a significant component of their pathogenic effects in type 2 immune responses.

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Involvement of cysteinyl leukotriene 2 receptors in airway allergic inflammation in mice

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Introduction: Cysteinyl leukotrienes (CysLTs) are a family of potent inflammatory lipid mediators and play an important role in asthma. Their actions are mainly mediated by CysLTs-selective receptors, $CysLT_1R$ and $CysLT_2R$. $CysLT_1R$ antagonists have now been widely used in the treatment of asthma. In contrast, recent studies demonstrate that $CysLT_2R$ negatively regulates the development of CysLT-dependent Th2 pulmonary inflammation using $CysLT_2R$-deficient mice, although the role of $CysLT_2R$ in asthma is not fully elucidated.

Objective: Therefore, we examined the effect of a $CysLT_2R$ antagonist on asthma-like phenotype in mice.

Methods: BALB/c mice were sensitized by intraperitoneal injections of crude extract of *Dermatophagoides farinae* (Der f) with alum, and then administered intratracheally Der f three times. Twenty-four hours after the final allergen challenge, airway responsiveness to acetylcholine was measured and bronchoalveolar lavage (BAL) was carried out.

Results: Treatment with a $CysLT_2R$ antagonist prior to the final allergen injection significantly inhibited airway hyperresponsiveness to acetylcholine, eosinophilic inflammation, Th2 cytokine and Th2-related chemokine production in the BAL fluid and histopathological changes in mice.

Conclusion: These findings demonstrated therapeutic potential for $CysLT_2R$ antagonists in the treatment of atopic asthma.

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The regulatory role of a novel activating receptor in mast cell- and IgE-dependent anaphylaxis

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Leukocyte mono-immunoglobulin-like receptor (LMIR) (also called CD300) belongs to a paired activating and inhibitory receptor family. LMIR3 (also called CD300f) is an inhibitory receptor which contains immunoreceptor tyrosine-based inhibitory and switch motif (ITIM and ITSM) in its cytoplasmic region. We have previously identified extracellular ceramide as a ligand for LMIR3 and demonstrated that ceramide-LMIR3 binding inhibits IgE-mediated mast cell activation and allergic responses (Immunity, 2012). On the other hand, we have also shown that LMIR7 (also called CMRF-35-like molecule-3), which is highly homologous with LMIR3 in an extracellular immunoglobulin-like domain, is an FcRγ-coupled activating receptor in mast cells (JBC, 2010). However, the physiological roles of LMIR7 have remained elusive.

Anaphylaxis, a clinical emergency, is often caused by the high-affinity IgE receptor (FceRI)-mediated mast cell degranulation. Here we describe a previously unappreciated role of LMIR7 in promoting FceRI-mediated anaphylaxis. Notably, we identified candidate lipids as ligands for LMIR7 by physical binding and functional reporter assays. Ligand lipid-LMIR7 binding alone did not induce degranulation in bone marrow-derived mast cell (BMMC), however it enhanced FceRI-mediated degranulation by amplifying FceRI signaling presumably due to the strong co-localization of ligand lipid-bound LMIR7 with cross-linked FceRI in BMMC. We confirmed that ligand lipid as well as ceramide were located in the surrounding of tissue mast cells. Importantly, IgE- and MC-dependent anaphylactic responses (passive systemic or cutaneous anaphylaxis responses) were attenuated by LMIR7 deficiency in mice or by inhibiting ligand lipid-LMIR7 interaction (e.g., pre-treatment with LMIR7-Fc, in which an extracellular domain of LMIR7 is fused to an Fc domain of hunan IgG1). In contrast, the same anaphylactic responses were enhanced by treating with ligand lipid-containing vesicles in wild-type mice. Thus, ligand lipid-LMIR7 binding efficiently primes tissue mast cells for enhanced degranulation upon FceRI engagement, leading to augmented anaphylactic reactions in mice.

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Basolateral sphingosine-1-phosphate stimulation influences barrier integrity of intestinal epithelial cells

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Background: The bioactive lipid sphingosine-1-phosphate (S1P), which is released by activated mast cells during allergic inflammation, plays a major role in lymphocyte trafficking and immune activation. Acting via one of its five G-protein coupled receptors (S1P1-S1P5), S1P was revealed as an important regulator of vascular as well as epithelial barrier function. Based on this knowledge, the aim of this study was to evaluate the effect of basolateral S1P stimulation on barrier integrity of intestinal epithelium.

Methods and Results: Well-differentiated Caco2 cells grown as tight monolayer were used as a model for human intestinal epithelium. S1P receptor as well as tight junction protein expression was determined using real-time PCR. The effect of basolateral S1P stimulation on transepithelial electric resistance (TEER) was measured as Ohm/cm^2 using Endohm Chambers. Real-time PCR evaluations demonstrated that well-differentiated CaCo2 cells express two of the five S1P receptors, S1P2 and S1P3. Basolateral stimulation with different concentrations of S1P (100nM and 500nM) induced regulation of mRNA expression of the tight junction proteins claudin-1, claudin-4, and zonula occludens-1. In addition, basolateral S1P significantly increased transepithelial electric resistance in a dose- and time-dependent manner.

Conclusions: Our data demonstrate that S1P has an important barrier protective function in human intestinal epithelial cells. We suggest S1P release by mast cells might represent a protective mechanism to increase intestinal epithelial barrier integrity during allergic inflammation.

Supported by the Austrian Science Fund FWF grant KLI284.

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Increased Expression of Filaggrin in Nasal Polyps as compared to Nasal mucosa of patients with allergic rhinitis

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Purpose: Chronic rhinosinusitis (CRS), with nasal polyposis (NP) is a chronic inflammatory disease of the upper airways often characterized by markedly increased numbers of eosinophils, Th2 cells,, and mast cells. Previously, we and others have shown increased expression of TSLP, periostin and increased number of IL-13+cells and reduced FoxP3+cells in NP. Epithelial-derived genes such as filaggrin have been shown to regulate barrier function and defects in their expression in atopic dermatitis (AD) have been reported . Here we investigated the expression of filaggrin in NP from atopics and non-atopics in comparison with the nasal mucosa of patients with allergic rhinitis (AR) and its potential role in NP.

Methods: Nasal polyp specimens and biopsies of nasal mucosa were obtained at surgery as part of the treatment for removal of NP or for hypertrophied turbinates. Immunoreactivity for filaggrin in NP from atopic and non-atopic patients and in the nasal mucosa of patients with AR was analyzed by immunohistochemistry using the peroxidase-based Avidin-Biotin Complex (ABC) method. Cell counts were analyzed using an objective micrometer and the density of immunoreactivity was quantified by Image J analysis system.

Results: Immunoreactivity for filaggrin was found mainly in epithelial cells and also some inflammatory cells in both nasal polyps and in the nasal mucosa of patients with AR. The number of Filaggrin+ cells in the epithelium of patients with NP was significantly greater than that in the nasal mucosa of the AR patients. The number of filaggrin+ cells in the lamina propria of patients with NP was also significantly grester than that in the nasal mucosa of the AR patients. Unlike our previous observations where atopic NP had higher expression of TSLP, periostin, IL-13, there was no difference in the number of filaggrin+ cells between nasal polyps from atopic and non-atopic patients.

Conclusions: Based on the present findings of an increased expression of filaggrin in NP irrespective of the atopic status, filaggrin may potentially play a role in the regulation of barrier function in nasal polyps.

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Analysis of transcriptional regulation for periostin

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Background: Periostin, an extracellular matrix protein, constitutively maintains tissue organization. It also plays important roles in the pathogenesis of allergic diseases such as bronchial asthma and atopic dermatitis as a matricellular protein at the downstream of the IL-13 signaling pathway; however, the transcriptional regulation of periostin remains undetermined.

Methods: We first investigated whether STAT6, a critical transcription factor of IL-13-mediated signaling, was involved in the IL-13–dependent periostin expression by STAT6 knockdown in a human fibroblast MRC5. We then narrowed down the critical amino acid sequence for IL-13-dependent periostin expression using HEK293T cells with reporter gene plasmids containing various truncated and/or mutated periostin promoter sequence. Moreover, we investigated whether *de novo* protein synthesis was necessary for IL-13–dependent periostin expression using cycloheximide.

Results: Knockdown of STAT6 almost completely diminished the periostin protein expression in MRC5 cells. Promoter truncation analyses showed that the region from -838 bp to -105 bp was important for the IL-13–induced response. The mutation analyses demonstrated that the TTC(N_3)GAA motif located within -137 bp to -129 bp was critical for the periostin expression by IL-13. Furthermore, treatment of MRC5 cells with cycloheximide decreased the IL-13–dependent periostin expression, suggesting requirement of *de novo* protein synthesis in response to IL-13.

Conclusions: We identified the critical role of STAT6 for IL-13–dependent periostin expression and its binding site on the human periostin gene. The binding site was not the typical TTC(N_4)GAA motif for STAT6 but a TCC(N_3)GAA motif, to which other STAT transcription factors usually bind.

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Educational programs in anaphylaxis: contents, methods, target groups

Johannes Ring and Knut Brockow

Anaphylaxis as the maximal variant of a immediate type hypersensitivity reaction represents the most dramatic emergency situation in allergy. Prevalence rates in the population are estimated between 1 and 3 %. There seems to be an increase, especially with regard to pollen-associated food reactions. In spite of great progress in the understanding of the pathomechanisms involved, there is a tremendous gap of information in affected individuals but also among non-specialists physicians, relevant groups of care-givers as well as in the general public and with political decision makers.

Following the example of the successful "Asthma" and "Eczema" schools a structured standardized and quality controlled educational program for "anaphylaxis school" was developed consisting of 2 x 4 didactic units (45 minutes) to be given in an interactive and interdisciplinary setting. In order to guarantee the didactic quality, train-the-trainer seminars have to be performed before trainers are entitled to do the actual educational program.

In a prospective randomized clinical trial the effect of this intervention was studied in 190 persons. There was a significant improvement not only in knowledge about anaphylaxis management but also in acute practical behaviour in handling the emergency medication together with improved quality of life (Brockow et al. 2015). The program is now performed in various parts of Germany and will be spread out to Austria and the German speaking parts of Switzerland. It is partly reimbursed by insurances. The standardized manual is constantly updated; trainers and teachers of train-the-trainer seminars undergo continuous quality control and supervision. A special program for children with anaphylaxis is under development. There are now over 80 anaphylaxis trainers in Germany and 8 "Anaphylaxis Academies" where train-the-trainer seminars are offered.

Recently it became obvious that not only affected patients have a need for better and more information but also other target groups. Therefore shorter seminars (one evening with 2 – 3 h) are offered to primary care physicians, nurses, nursery nurses, teachers, pharmacists, paramedics. It seems essential that these educational activities are not classic "frontal" lectures but interactive in nature and combined with practical exercises. Evaluation of these educational events shows the need for more and better information transfer in the field of anaphylaxis.

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Increased plasma concentrations of angiogenic and lymphangiogenic factors in patients with hereditary angioedema with C1-INH deficiency

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Background: Hereditary Angioedema with C1 Inhibitor Deficiency (C1-INH-HAE) is a rare inherited genetic disease clinically characterized by recurrent acute swelling episodes of the skin, gastrointestinal tract and upper airways resulting from increased vascular permeability. Reduced activity of C1-INH may result in an instability of kinin pathway with the generation of bradykinin resulting in increased vascular permeability. Bradykinin increases the release of nitric oxide and Vascular Endothelial Growth Factor (VEGF) from endothelial cells. VEGF, was originally described as vascular permeability factor (VPF). The Angiopoietin-Tie receptor system (Ang-Tie) is also essential for endothelial cell maturation. Ang1 and Ang2 are released at sites of inflammation and/or angiogenesis. Angs and VEGFs regulate vascular permeability. The pathogenesis of C1-INH-HAE is not completely elucidated. The aim of this study was to analyze the plasma concentrations of VEGFs and Angs in patients with C1-INH-HAE.

Methods: 68 healthy controls and 128 C1-INH-HAE patients were studied. Concentrations of angiogenic (VEGF-A, Ang1, Ang2), anti-angiogenic (VEGF-A_{165b}) and lymphangiogenic (VEGF-C) factors were evaluated by ELISA. Functional assay of C1-INH was assessed by EIA kit.

Results: Plasma concentrations of VEGF-A, VEGF-C, Ang1 and Ang2 were higher in C1-INH-HAE patients in remission than in controls. The anti-angiogenic isoform, VEGF-A_{165b}, did not differ between the 2 groups. Plasma concentration of VEGF-A are correlated to the decrease of functional activity of C1-INH. Interestingly, a correlation was found between the number of attacks and both VEGF-A, VEGF-C and Ang2 concentrations.

Conclusions: The results of this study indicate that the plasma concentrations of several angiogenic/lymphangiogenic factors that alter vascular permeability are increased in patients with C1-INH-HAE in remission. This condition might predispose to angioedema attacks. The cellular sources of this angiogenic/lymphangiogenic factors in these patients with C1-INH-HAE are under investigation.

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Condition of hypoxia, cytokine or estrogen stimulation of endothelial cells augments activation of the surface-bound prekallikrein-high molecular weight kininogen complex and releases urokinase: Implications for hereditary angioedema (HAE).

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Background: When the prekallikrein-high molecular weight kininogen complex (PK-HK) is bound to endothelial cells, prekallikrein is stoichiometrically converted to kallikrein due to release of heat shock protein-90 (Hsp90).

Rationale: Since attacks of hereditary angioedema can be related to stress, infection or estrogen we questioned whether hypoxia, estrogen or cytokine stimulation of endothelial cells could augment release of Hsp90 and prekallikrein activation. We also tested release of profibrinolytic enzymes, urokinase (UK) and tissue plasminogen activator (TPA) since HAE with normal C1 inhibitor is associated with markedly diminished levels of plasminogen activator inhibitor 2 (PAI-2) regardless whether a factor XII mutation is present or not.

Methods: Cells were stimulated with agonists and Hsp90, UK, and TPA were measured in the culture supernatants by ELISA. Activation of the PK-HK complex was measured employing pro-phe-arg-p-nitroanilide reflecting kallikrein formation.

Results: Hsp90 release was stimulated with optimal doses of H_2O_2 (500 μM to induce hypoxia) estradiol, IL-1, and TNFα (10ng/ml) from 15 min to 120 min. TPA release was not augmented by any of the agonists tested but UK was released by IL-1, TNFα and thrombin (positive control) but not estrogen. Augmented activation of PK-HK was seen with each agonist that releases Hsp90. Addition of 0.1 molar factor XII relative to PK-HK leads to rapid formation of kallikrein; factor XII alone does not autoactivate.

Conclusions: Interleukin-1, TNFα, and estrogen stimulate release of Hsp90 and augment activation of the PK-HK complex. IL-1 and TNFα stimulate release of urokinase which can augment fibrinolysis.

Clinical Implication: Attacks of angioedema in patients with HAE may be initiated by hypoxia or use of estrogen or infection. Cytokine or estrogen stimulation of endothelial cells and activation of the PK-HK complex may contribute to this process while release of urokinase is particularly germane when levels of PAI-2 are diminished.

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Idiopathic Angioedema: Difficult Cases and Uncommon Findings

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Background: Angioedema is defined as the swelling of subcutaneous tissue due to vascular leakage. Steroid and antihistamine non-responsiveness may indicate a possible complement defect. In the absence of allergies and other identifiable causes, the recurrent angioedema cases are classified as "idiopathic". We investigated two cases of recalcitrant angioedema using whole exome sequencing.

Methods/Results: The first case is a 17 year old female who presented with recurrent swelling of her face, hands, and feet, headaches, and severe abdominal pain not responsive to steroids, antihistamines, C-1 esterase inhibitor, fresh frozen plasma, and bradykinin receptor inhibitors. Whole Exome Sequencing (WES) identified compound heterozygosity for the E172K and G216R variants in the tyrosine kinase with immunoglobulin- like and EGD like domains 1 (TIE-1) gene. In a murine model, mutations in TIE-1 gene result in vascular leakage and affect lymphocyte development. In addition to the angioedema, our patient also presented with B cell lymphopenia, suggesting a functional correlation of the genetic findings.

The second case is a 20 year old female with a two year history of recurrent and severe swelling of her lips and tongue. This patient also did not respond to any standard HAE therapies like the patient in the first case. She also had a normal complement work-up. WES showed she is heterozygous for a variant of the Phosphodiesterase 11A (PDE11A) and Histidine Rich Glycoprotein (HRG) gene. In vitro studies with this patient's serum showed increased cytotoxicity against fibroblasts and endothelial cells as well as abnormal protein size of HRG on western blot.

Conclusion: These two cases suggest that angioedema can result from either structural defects of the vascular endothelium or soluble factors that impact on the endothelial cells. Further understanding of the endothelial cell and immune interactions will be important to better understanding of mechanisms of what is known as idiopathic angioedema.

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Sustained Effect and Clinical Outcomes in Chronic Spontaneous Urticaria in Patients Receiving omalizumab for Several Years

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Rationale: We don't have studies exploring the natural course of the disease and OmAb control for several years.

Objective: To analyze the clinical outcome and patterns of response in patients with Chronic Spontaneous Urticaria (CSU) treated with omalizumab (OmAb) for more than three years.

Methods: We analyzed the data of 15 patients treated with OmAb for 3 to 7 years (average of 4.8±1.3 years). We assessed the Urticaria Control Test (UCT), Urticaria Activity Score average of seven days (UAS7) in 2012 and in 2015 plus the Quality of life specific for CSU (CU-Q2OL).

Results: Out of the 15 patients, 6 went on complete remission after second, third or fourth dose. One patient received 5 OmAb doses and 1-year cyclosporine due to the not-reimbursement of OmAb. None of the patients who received OmAb for several years lost response with time. Two patients went on remission for 5 years but have to reintroduce OmAb due to CSU relapse, both patients showed the same good response to OmAb. 3 patients received a monthly administration and the rest from every 6 to 14 weeks. We did not find any significant differences in UCT, UAS7, or CU-Q2OL when comparing monthly vs. "on demand" administration. All the patients received 300mg except for 2 patients who went to 150 due to good response. These two showed worse scores prior to the administration of OmAb (UCT = 0, UAS7=32, CU-Q2OL=79) and are scheduled to receive 300mg thereafter. Only 2 patients needed additional medication (Rupatadine 20mg/day levozetiricine 5mg/day respectively). The rest of the patients had a UCT=20, UAS7=0, CU-Q2OL= 0. One of the patients stopped receiving OmAb due to non-reimbursement with UCT=20 to 0, UAS7=0 to 32 and CU-Q2OL from 0 to 68.

Conclusions: In our study, 40% of patients went on remission upon OmAb treatment. OmAb maintains a complete CSU control response along several years and is also effective on reintroduction. Clinical severity and quality of life scores are similar when administering OmAb on a regular basis or upon appearance of symptoms.

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Effects of an oral CRTH2 antagonist on CRTH2-bearing blood leukocytes in chronic spontaneous urticaria patients

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Background: Skin mast cell (MC) activation and degranulation contributes to the recruitment of lymphocytes, eosinophils and basophils in skin lesions in chronic spontaneous urticaria (CSU). Activated MCs release prostaglandin D2 (PGD2), which binds to the CRTH2 receptor, leading to the recruitment and activation of leukocytes. Levels of CRTH2 are reduced on blood basophils and eosinophils in patients with CSU supporting that this pathway is engaged. We examined the effects of an oral CRTH2 antagonist, AZD1981, on CRTH2-bearing leukocytes in CSU patients.

Method: Antihistamine refractory adult CSU subjects were enrolled in a study involving 4 weeks of double-blind, placebo-controlled treatment with AZD1981. Subjects recorded daily symptoms of hive and itch and underwent blood sampling for CBC with differential, PGD2-induced eosinophil shape change assay, total blood leukocyte histamine content (TLHC), and whole blood flow cytometry at baseline, end of treatment, and 2 weeks after the end of treatment.

Results: 36 subjects entered screening and 22 subjects completed the full treatment phase. A marked reduction in PGD2 induced eosinophil shape change was noted in 12 subjects at the end of treatment relative to baseline and due to active therapy with AZD1981. Peripheral blood eosinophil percentage also rose in these subjects (3.17 % to 4.43 % n= 12, p=. 03) as did blood basophil percentage (0.48% to 0.64 %, n=10, p< .01). However, TLHC was not increased at the end of treatment, but was significantly lower 2 weeks after the end of treatment relative to baseline (19.34 vs. 26. 31 ng/cc, n=12, p < .03).

Basophil surface CRTH2 expression increased 30% at the end of treatment (235 to 304 net Mean Fluorescence Intensity units (MFI), n=12, P < .02). CRTH2 levels fell 2 weeks after treatment but were still above baseline (p < .05). No similar change was noted in eosinophil CRTH2 expression.

Conclusions: CRTH2 antagonist use in active CSU patients results in blunting of PGD2 induced eosinophil shape change, a rise in blood eosinophils and basophils, and altered basophil CRTH2 expression. This supports a reduction in basophil and eosinophil recruitment to the skin.

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Sensitization to mouse (Mus m 1) is a leading pattern in Amaxhosa atopis dermatitis patients in Cape Town region, South Africa.

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Background: The prevalence of Atopic dermatitis on (AD) is on the rise in some African countries. Data on the prevalence, atopic characteristics and sensitization patterns in AD patients from Africa are sparse.
Thus we wanted To define the prevalence of raised IgE and sensitization patterns to different allergens in an African population with AD

Method: Subjects (n=102) with AD of Xhosa ethnic background attending a tertiary hospital were recruited. These patients were then matched for age, sex and ethnicity and compared with 155 healthy controls. The subjects had bloods taken for IgE levels (both total and specific) and for parasite serology. ImmunoCAP® ISAC wasused to determine specific IgE against 103 allergens.
ImmunoCAP/RAST was used to compare the ISAC findings for HDM, peanut and egg white.

Results: The atopic eczema severity score according to NESS (Nottingham eczema severity score) showed 23(22.5) with mild,, 45(44.1%) with moderate and 34(33.3%) with severe AD. The total IgE was raised in 91.8% of the 73 patients compared to 48.7% of the 148 controls whose samples were available for testing. Of the 73 patients,28% had levels >5000 compared to 0.6% of 148 controls.Higher IgE levels were found in more severe disease. A positive specific IgE to at least one allergen was found in 89.5% of the patients (n=76) compared to 20.3% of controls(n=148). The most common inhalant allergens to which the patients were sensitized were: house dust mite, storage mites, grass, and pollen while egg proteins were the highest prevalent food allergens.A striking finding was the high sensitization rate to mouse allergen (Mus m 1), correlating to a certain degree inversely with the socioeconomic status, as patiets in low-income households were more often sensitized to Mus m 1.

Conclusion: As in other populations, IgE levels in the Xhosa population are also higher in AD patients compared to controls and correlate to clinical severity. Sensitization patterns in AD patients are dominated by mites and grass,and especially in childhood also to foods (egg white, peanut). As found in patients with asthma in underprivileged tropical or inner-cities areas , sensitization against mouse (Mus m1) was predominant finding in the AD patients. Thus sensitization to Mus m1 seems to be a leading leading marker namely for patients with lower income also in AD.

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Prevalence of mediator-related symptoms in patients with mastocytosis and response to anti-mediator treatments

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Background: Mastocytosis is a rare disease characterized by clonal proliferation of mast cells in the skin and other organs, such as bone marrow, liver, spleen, gastrointestinal tract and lymphnodes. Symptoms due to the release of mast cell-derived mediators constitute the main clinical picture of mastocytosis, either cutaneous or systemic. Given the benign course of the disease in the majority of patients with mastocytosis, the primary therapeutic target is to control mediator-related symptoms.

Methods: We evaluated the prevalence of mediator-related symptoms and the requirement for anti-mediator treatment in 90 patients (36 males, median age 31 years, range 1-74). Grading of symptoms was 0: no symptoms; 1: mild, infrequent, no continuous treatment required; 2: mild/moderate, frequent, kept under control with daily therapy; 3: severe, frequent, difficult to control with daily or combination therapy; 4: required immediate therapy and hospitalization (Valent *et al.* Eur J Clin Invest 2007:37:435). Serum tryptase was measured by ImmunoCAP (Phadia).

Results: The most frequent clinical manifestations (63% of patients) were skin symptoms (urticaria, pruritus, angioedema and flushing) followed by osteoporosis (39%), which was only present in adult patients. About one third of patients (26/90) had no or minor symptoms and did not require treatment. The majority of patients (55/90) required only anti-H_1antihistamine either on-demand (40/90) or as continuous treatment (15/90). More than one drug, including the association of two antihistamines or other combinations (anti-H_1 plus anti- H_2 and/ or oral cromolyn sodium) was required in 10% of patients (9/90). Serum tryptase was higher in patients with severe symptoms (grading 2 or 3) than in those with mild (grading 1) or no symptoms (grading 0) (75.6 *vs.* 21.1 ng/ml; $p<0.05$)

Conclusions: Most patients with mastocytosis have mediator-related symptoms and they require either on demand or continuous pharmacological treatment. Histamine is the main mediator responsible for symptoms since the majority of patients are responsive to antihistamines. Higher levels of tryptase are associated with severe mediator-related symptoms.

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Barriers to the use of human tissue in asthma research

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Much asthma research is carried out in non-human models, however it can be difficult to translate findings from these to efficacious therapies. As asthma is a disease unique to humans, the development and application of human tissue-based approaches with which to study the disease should be considered a priority.

The NC3Rs, working with Asthma UK, the UK Respiratory Research Collaborative (UKRRC) and the UK Human Tissue Authority (HTA) surveyed the UK asthma research community to capture information on:

- The way human tissue is currently used in asthma research;
- The level of knowledge surrounding the regulatory requirements and guidance on human tissue use;
- The barriers to wider uptake of human tissue-based approaches in asthma research.

The online questionnaire was completed by 59 scientists active in asthma research from academia, pharmaceutical companies, SMEs and the NHS.

The survey indicated widespread use of human tissue in asthma research with 86.4% of respondents using human tissue in some capacity, but highlighted discrepancies between the type of tissue currently used and what researchers would like to use. The impacts of wider adoption of human tissue approaches could be far-reaching; with the majority of respondents agreeing that increased use of human tissue would improve our understanding of the pathobiology of asthma (93.4% of respondents), speed up the development of efficacious new therapies (88.4%) and reduce animal use in asthma research (75.1%). However the survey highlighted a number of barriers to further uptake including the availability of both normal and diseased tissue, regulatory concerns, and practical issues associated with the acquisition and storage of human tissue.

The NC3Rs is leading a programme of work to address these barriers, involving the production of a web resource to support researchers in accessing and using human-tissue. We are currently working with respiratory disease researchers, regulators and health authorities to share experiences, increase access to tissue, simplify the regulatory framework and support the wider adoption of human tissue-based approaches for respiratory disease research.

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Innovative Nasal Filters Allow For Allergen Exposure Monitoring and Are Acceptable To Wear

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Background: For over 25 years the examination of environmental allergens has shaped our understanding of the role of allergen exposure in the development of allergic disease. These studies have highlighted the important role allergens play in allergic sensitisation and exacerbation. The most common sample type analysed for monitoring allergen exposure is settled dust. Settled dust is an easily available source which yields lots of allergen. However, this sample type is only a snap shot of the allergen reservoir and may not take into account the full spectrum of allergen which a subject breathes in during their whole day. In this study, we sought to assess the feasibility of using a new nasal filter for the assessment of allergen exposure.

Methods: The nasal filter consists of a membrane that removes particles by means of interception and impaction. Volunteers wore the nasal filter for up to 24 hours during their normal daily routine. For comparison settled dust was collected from each volunteer's home. Allergen was extracted from nasal filters and settled dust by gentle rocking in phosphate buffered saline with tween for two hours. The levels of ten major allergens captured by these sampling methods were quantified using a multiplex array for quantification of indoor allergens (MARIA). Finally, in a randomized control trial the device was evaluated on usability and tolerance.

Results: Significant levels of allergens were readily detectable in the nasal filter extracts (up to 0.5ng per filter). These included allergens from house dust mite (Der p 1), dog (Can f 1) and pollen (Bet v 1). There was some correlation with corresponding samples collected from settled dust. We found that most people (90%) seemed to quickly (within 60 minutes) forget that they were wearing the nasal filter. Most (85%) did not experience a difference in breathing resistance.

Conclusions: These data indicate that nasal filters may be considered a simple and easily wearable method for monitoring allergen exposure. This sampling method which takes into account a wider spectrum of potential allergen exposure sources may improve our understanding of the role of allergens in the development of allergic disease.

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Identifying Immune deficiencies by Curbside Consultation; iCurb

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Background: Immune deficiencies (IDs) affect approximately 1 in 1200 persons in the United States. Diagnosis is delayed over 10 years in 45% of the cases. We hypothesized that physicians lack understanding of immune disorders, the components of an immune work-up, and its interpretation are the three main obstacles in identifying IDs.

Methods: We designed a system called curbside consultation (iCurb) and tested it on a large number of physicians and patients in Northern Virginia, with 2.8 million residents (about a third of the state). We surveyed 328 primary care physicians and specialists asking the number of ID patients they have diagnosed in their practices based on a computerized search of ICD-9 codes, in a database of over 1 million patients. We then provided them with the 10 warning signs of immunodeficiency and offered to perform an immune work-up that consisted of assaying all components of the immune system in a qualitative (e.g. vaccine responses), quantitaive (e.g. CBC with differential, serum antibody titers, detailed phenotyping of T, B and NK cells) as well as input/output assays (e.g. Th17 upon invitro stimulation). We set up assay systems that would bring down the total cost of the immune phenotyping and input/output assays to less then $100 in total. Patients' clinical history and exam findings were reported to us on an i-Curb form along with a blood sample to perform the immunological studies.

Results: A total of 9265 patients were involved in the study. Prevalence of IDs increased to 58:100,000 from 5.3:100,000 with the curbside consultation ($P < 0.001$). The most significant change was observed in otolaryngology, pulmonary, and pediatric gastroenterology patients. If we were to apply step-wise approach to immune testing, assaying CBC and serum antibodies alone would detect only up to 30% of the IDs. Each component of iCurb had a statistically significant addition to the identification of IDs.

Conclusion: Offering a pre-set diagnostic tool, along with interpretation can be a cost effective solution to identifying IDs and avoiding the need for a stepwise approach. We call this method i-Curb and offer testing it in larger populations for validation.

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Bleach Baths Improve Atopic Dermatitis, Reduce Itch and Repair Barrier

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Background: Studies have shown that bleach baths improve disease severity in subjects with atopic dermatitis (AD). This open label study was designed to test whether bleach normalizes skin barrier function and/or reduces itch in adults with AD who are colonized with *S. aureus*.

Methods: AD and nonatopic, healthy controls (NA) subjects were instructed to take two bleach baths (0.005% NaClO; 5-10min duration) per week for a total of 12 wks as add-on therapy. Skin barrier assessments included pH, stratum corneum (SC) hydration, transepidermal water loss (TEWL), which was measured in nonlesional skin. Efficacy measures included EASI, 5D pruritus, and Itchy QoL. All measures were done at 0, 6 and 12 wks.

Results: Interim analysis of the results from 9 AD (7M/2F; 45 ± 15 yrs [Mean ± SEM]) and 4 NA (2M/2F; 46 ± 3 yr) was performed. A decrease in EASI score of 43 ± 10% (P=0.006) was observed, with 44% of subjects achieving an EASI50 at 12 wks. TEWL was greater in AD subjects compared to NA (P=0.03) at baseline and TEWL decreased by 25 ± 8% in AD subjects after 6 wks of treatment (P=0.008). Surface pH became more acidic within 6 wks of bleach baths (P=0.055; 12 wks P=0.05). There was no improvement observed in SC hydration. Itch was significantly reduced as measured by ItchyQoL (22 ± 6%; P=0.002) and 5-D Pruritus (21 ± 5%; P=0.002) after 12 wks of treatment. Six out of 9 AD subjects remained *S. aureus* culture positive after bleach treatment.

Conclusions: In summary, adjunct treatment with bleach baths improved disease severity, skin barrier function and pruritus in adult AD subjects. Additional analysis will be performed when enrollment is completed (n=15 AD) and will include serum biomarkers, skin microbiome and transcriptome.

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Omalizumab reduces bronchial mucosal inflammation and improves lung function in non-atopic asthma

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Background/Objectives: Control of severe asthma remains a challenge, particularly in non-atopic patients who are currently denied anti-IgE therapy which is perceived as ineffective. We have utilised a placebo controlled study to demonstrate that omalizumab therapy reduces bronchial inflammation and consolidate proof of principle that it can improve lung function in non-atopic asthmatics.

Methods: 16 symptomatic, non-atopic asthmatics were randomised (1:1) to receive omalizumab or identical placebo treatment for 20 weeks. Inflammatory cells were enumerated in sections of bronchial mucosal biopsies collected before and after 14-16 weeks of treatment, following which the patients were destabilised by substantial, supervised reduction of their regular anti-asthma therapy. Lung function (FEV_1), asthma-related symptoms (Juniper ACQ) and quality of life (Juniper mini-AQLQ) were monitored.

Results: Omalizumab, compared with placebo therapy, was associated with significant median percentage reductions in the numbers of bronchial mucosal total IgE^+ cells ($p<0.001$), mast cells ($p<0.001$) and plasma cells ($p=0.005$). IgE^+ mast cells were also reduced but not significantly. Mucosal B lymphocytes and eosinophils were not altered. By 20 weeks, lung function had declined in the placebo treated patients but improved in the omalizumab treated patients, with significant differences in absolute ($p=0.02$) and % predicted FEV_1 ($p=0.009$).

Conclusions: Omalizumab reduces bronchial mucosal inflammation and can improve lung function in non-atopic asthmatics. A policy of restricting therapy to asthmatics with the "endotype" of atopy as conventionally defined may exclude potential responders.

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Omalizumab is effective and safe in dermographic and cold urticaria

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Background: Dermographic and cold urticaria, two frequent forms of physical urticaria, are often chronic, debilitating, and resistant to antihistamines, the first line treatment. Case reports suggest that omalizumab, an anti-IgE antibody licensed for asthma and chronic idiopathic/spontaneous urticaria, may be effective. As of now, there are no controlled trials with omalizumab in dermographic urticaria, cold urticaria, or any other form of physical urticaria.

Methods: We conducted two multicenter, randomized, double-blind studies, to evaluate the efficacy and safety of omalizumab in H1-antihistamine resistant patients with dermographic urticaria (DermU) or cold urticaria. 62 DermU patients and 31 ColdU received three subcutaneous injections, spaced 4 weeks apart, of omalizumab at doses of 150mg or 300mg, or placebo, followed by an 8-week observation period. The primary efficacy outcome was the change in trigger thresholds from baseline to week 10 assessed with FricTest (in DermU) and TempTest (in ColdU).

Results: Baseline provocation thresholds were comparable in all treatment groups of both trials. At week 10, the mean (±SD) change of trigger thresholds in DermU was -0.6±1.4 in the placebo, -1.8±1.7 in the omalizumab 150mg ($P<0.05$), and -2.0±1.8 in the omalizumab 300mg group ($P<0.01$). In ColdU patients, changes of trigger thresholds from baseline to week 10 were -0.3±3.9 in the placebo, -10.6±7.6 in the omalizumab 150mg ($P<0.001$), and -10.4±9.4 in the omalizumab 300mg group ($P<0.05$). The rates of DermU and ColdU patients who showed complete response were 11% and 0% in the placebo, 44% and 40% in the omalizumab 150mg, and 53% and 44% in the omalizumab 300mg group, respectively. The frequency of adverse events was similar across groups. The frequency of serious adverse events was low in both trials, with one event in the 300mg group and one in the placebo group, both in DermU patients.

Conclusions: Omalizumab reduced provocation trigger thresholds in DermU and ColdU patients and resulted in high rates of patients with complete response. (NCT02169115 and NCT01580592).

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Inhaled budesonide induces expression of genes involved in transcription and signalling in human airways: a randomized controlled trial

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Background: Inhaled corticosteroids (ICS) are the cornerstone of asthma therapy, and exert their anti-inflammatory effects by either repressing inflammatory gene transcription (*transrepression*) or enhancing anti-inflammatory gene expression (*transactivation*). However, understanding their anti-inflammatory actions and *in vivo* effects remains incomplete, and it is unclear whether clinically relevant doses of ICS result in transactivation *in vivo* in human subjects. The objective of this study was therefore to characterize corticosteroid-induced changes in gene expression in human airways following a single, clinically relevant dose of inhaled budesonide.

Methods: Twelve healthy male subjects were randomized to a prospective, double-blind, placebo-controlled, two-period cross-over study. Six hours after a single dose (1600 µg) of inhaled budesonide or placebo, peripheral blood was collected and endobronchial brushings and biopsies were obtained via bronchoscopy for real-time PCR and/or microarray analysis of gene expression.

Results: Inhaled budesonide resulted in significantly increased expression of multiple genes in the biopsy samples, including DUSP1, FKBP5, NFKBIA, RGS2, TSC22D3 and ZFP36. With the exception of *RGS2*, these mRNAs were also up-regulated in epithelial cells obtained from bronchial brushings. Following microarray analysis, stringent (≥2 fold, 5% false discovery rate) or less stringent (≥1.25 fold, P≤0.05) criteria identified 46 and 588 budesonide-induced genes, respectively. Transcription factors, receptors and signaling genes represented ~2/3 of each group. Gene ontology (GO) terms for transcription, signaling, metabolism, proliferation, inflammatory responses and cell movement were significantly associated with these genes, and the most enriched functional cluster included terms for positive regulation of proliferation, locomotion, movement and migration. GO terms for negative regulation of transcription and gene expression also correlated with the corticosteroid-induced genes, and with the transcriptional repressors TSC22D3 and ZBTB16 being highly induced, is consistent with the repressive effects of corticosteroids.

Conclusion: A single, clinically relevant dose of inhaled budesonide induced expression of multiple genes *in vivo,* including the novel transcriptional repressor, ZBTB16, that reduce inflammatory signaling and gene expression and may contribute to corticosteroid efficacy. These data provide new insights into the anti-inflammatory actions of corticosteroids, which, combined with the demonstration of numerous positive effects on transcription and signaling, should aid strategies to rationally improve corticosteroid clinical efficacy.

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Preventive and therapeutic amelioration of food allergy by inhibitors of histamine-releasing factor

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Food allergy with increasing prevalence is a major public health concern. Histamine-releasing factor (HRF) can stimulate histamine release and IL-4 and IL-13 production from IgE-sensitized basophils and mast cells. Little is known about potential involvement of HRF in food allergy. Diarrhea and Th2-type intestinal inflammation were induced by repeated ovalbumin (OVA) gavages in OVA-immunized BALB/c mice, accompanied by increased mast cell activation and higher HRF-reactive IgG levels. HRF inhibitors that block HRF-IgE interactions were pretreated before each OVA challenge to test whether HRF inhibitors prevent or ameliorate diarrhea and intestinal inflammation. HRF inhibitors were also administered before further gavages to diarrheic mice to test whether blockade of HRF-IgE interactions can reduce diarrhea occurrence and inflammation. Confocal microscopy revealed that orally administered HRF inhibitors preferentially target mast cells in the jejunum. Both prophylactic and therapeutic administration of HRF inhibitors reduced diarrhea occurrence, intestinal inflammation, and reduced mast cell activation in this model of food allergy. Relevance of the mouse data was studied by measuring serum levels of HRF and HRF-reactive IgE and IgG before and after oral immunotherapy of human patients allergic to hen eggs. Food allergy patients had higher plasma levels of HRF-reactive IgE and IgG than healthy controls, while HRF levels were similar between the two groups. The patients' HRF-reactive IgE and IgG levels were lowered within one week after the initiation of oral immunotherapy. However, the patients, who exhibited high sensitivity to allergen within 2 weeks of allergen avoidance after 12 months of maintenance immunotherapy, had higher HRF-reactive IgE, but not HRF-reactive IgG, levels than their levels one week after the initiation of oral immunotherapy. Therefore, HRF promotes diarrhea development and intestinal Th2 inflammation in mice and plasma levels of HRF-reactive IgE might serve as a biomarker for the long-term success of oral immunotherapy of human food allergy.

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Gauging Response in Allergic Rhinitis to Sublingual and Subcutaneous Immunotherapy (GRASS): Nasal Allergen Challenge and Local and Systemic Immunologic Responses

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Rationale: 3 years sublingual (SLIT) and subcutaneous (SCIT) immunotherapy have independently been shown to induce long-term benefits for 2-3 years after their discontinuation. However, there have been few head-to-head comparisons.

Methods. In a double-dummy DBPC single centre trial in moderate-severe seasonal rhinitis (n=106 randomised, mean age 33 yr, 70% male, withdrawal rate <5%/yr), we compared 2 yr treatment with SCIT (Alutard SQ, *Phleum pratense*, ALK Denmark, monthly injections) with SLIT (Grazax, ALK Denmark, daily tablets). Total nasal symptom scores (TNSS) and Th2 cytokines in nasal fluid were measured at 0-10 hr after NAC before and at 1 and 2yr during treatment and at 3yr, 1 yr after stopping treatment. Primary outcome was area under curve (mean AUC/hr for early [0-60 min] and late [1-10hr]) TNSS and primary endpoint SLIT v PL at 3yr. Grass pollen specific tetramer+ and CD154+CRTH2+ cells, serum-specific IgE, IgG4 and inhibitory activity for IgE-FAB were also measured. Analysis was by ANCOVA with correction for baseline yr.

Results. SCIT was effective earlier (TNSS: 34% < PL, p<0.001) and more effective (29% < SLIT, p=0.03) than SLIT at 1yr. Both SCIT (41.6% <PL, p<0.001) and SLIT (27.0% <PL less, p=0.02) were effective at 2yr. There was a trend for persistent decreases for SCIT (14.9% <PL, p=0.096) but not SLIT (5.6% <PL, p=0.62) at 3yr. Increases in Interleukin (IL)-4, IL-13 IL-5, and IL-2 in nasal fluid at 3-10 hr were inhibited equally (all p<0.005) after SCIT and SLIT at 2yr but not at 3yr. Circulating CRTH2+Tetramer+ and CRTH2+CD154+ cells were decreased at 2yr for SCIT (<PL, p<0.01) but not at 3yr. SIgE was increased for SLIT v PL (p<0.05) at 1 yr. Increases in serum sIgG4 and inhibition of IgE-FAB (SCIT and SLIT <PL, all p<0.05) were greater for SCIT (SCIT v PL, p<0.001) and persisted (SCIT and SLIT < PL, all p<0.05) at 3yr.

Conclusion. Both SCIT and SLIT suppressed allergen-induced nasal symptoms at 2yr. The effect of SCIT came on earlier and was greater than SLIT at 1yr. There was consistent suppression of clinical and immunologic responses at 2y but not at 3yr, one yr after discontinuation.

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Circulating CD4$^+$CXCR5$^+$PD-1$^+$FoxP3$^+$ T Follicular Regulatory cells are decreased in Allergic Rhinitis and restored following Subcutaneous and Sublingual Immunotherapy

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Background: Circulating T follicular regulatory cells (CD4+CXCR5+PD1+FOXP3+, Tfr) are a recently discovered specialised subset of effector T regulatory cells. Tfr cells exert their suppressive function via CTLA-4-dependent mechanisms. We hypothesised that Tfr cells are reduced in patients with seasonal allergic rhinitis (SAR) compared to non-atopic controls (NA). Furthermore, allergen immunotherapy (AIT) administered either by subcutaneous (SCIT) or sublingual (SLIT) route is associated with the induction of Tfr cells which may play a role in restoring immune tolerance.

Method: In a prospective controlled cross-sectional study of AIT, peripheral blood mononuclear cells were obtained from SAR (n=13), NA (n=13), SCIT (n=10) and SLIT (n=8). Circulating CD4+CXCR5+PD-1+FoxP3+ and CTLA-4+ Tfr cells were enumerated by flow cytometry. Additionally,IL-4+IL-21+CD4+CXCR5+PD-1+ICOS+ T follicular helper cells (Tfh) were enumerated. IL-4 and IL-21 levels were quantified in nasal fluid by Luminex MagPix assay before and 6-8 hr after nasal grass pollen challenge.

Results: Total nasal symptom scores were lower in SCIT and SLIT (p=0.001; p=0.01) compared to SAR and NA exhibited no symptoms after nasal challenge. The frequency of circulating CD4$^+$CXCR5$^+$PD-1$^+$FoxP3$^+$ and CTLA+ Tfr cells were lower in SAR compared to NA (p=0.01, p<0.001) and elevated in SCIT (p=0.007; p=0.004) and SLIT treated subjects (p=0.03; p=0.004). We confirmed that ICOS+ Tfh and IL-4+, IL-21+ Tfh cells were higher in SAR compared to SCIT (p<0.05), SLIT (p<0.05) and NA (p<0.05). Interestingly, IL-4 and IL-21+ levels were increased in SAR but not SCIT, SLIT and NA after allergen challenge (all, p<0.05). The proportion of Tfr cells correlated inversely with ICOS+ (Spearman; r= -0.32, p=0.03), IL-4+ (r= -0.33, p=0.03), IL-21+ (r=- 0.44, p=0.004) and IL-4+IL-21+Tfh cells (r=-44, p=0.004).

Conclusion: For the first time, we demonstrate that circulating CD4$^+$CXCR5$^+$PD-1$^+$FoxP3$^+$ Tfr cells are decreased in allergic rhinitis and restored following successful SCIT and SLIT.

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Pathogenic Th2 (Tpath2) cells in airway inflammation

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To develop more effective vaccines and strategies to regulate chronic inflammatory diseases, it is important to understand the mechanisms underlying the generation and maintenance of immunological memory. In 2011, we identified a highly pathogenic IL-5-producing memory Th2 cell subset in allergic airway inflammation (Endo et al. Immunity, 2011). Based on these data, we propose a new model called "Pathogenic Th population disease induction model" in the pathogenesis of Th1/Th2/Th17 diseases (Endo et al. Trends in Immunology, 2014). We have extended our research, and found that the pathogenic Th2 cells (Tpath2 cells) are a distinct cell population generated in vivo, and express high levels of IL-33 receptor component, ST2. We have investigated how Tpath2 cells are induced, and found that interleukin-33 (IL-33)-ST2 signals play a crucial role and selectively licenses memory Th2 cells to induce allergic airway inflammation via production of IL-5, and that the p38 MAP kinase pathway is a central downstream target of IL-33-ST2 in both mouse and human memory Th2 cells. In addition, we found that IL-33 induced upregulation of IL-5 by human memory CD4$^+$ T cells isolated from nasal polyps of eosinophilic chronic rhinosinusitis patients. Therefore, IL-33-ST2-p38 signaling appears to directly instruct memory Th2 cells to become Tpath2 cells that produce huge amount of IL-5 and induce eosinophilic inflammation in the airway. These newly identified memory type Tpath2 cells are CD44+ CD62Llo CXCR3lo CCR4+ CCR8+ IL-7Rα+ ST2+ CD4 T cells.

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Negative feedback mechanisms for ILC2 functions and type 2 innate immune responses.

Shigeo Koyasu

Recent studies have identified novel lymphocytes that do not express Rag-dependent antigen specific receptors but are able to produce large amounts of cytokines, collectively called innate lymphoid cells (ILCs). ILCs are classified into three groups based of their cytokine expression patterns. Group 1 ILC including NK cells and ILC1 produce IFN-γ in response to IL-12 and IL-18. Group 2 ILC (ILC2s) including natural helper cells, nuocytes and innate helper type 2 (Ih2) cells produce type 2 cytokines such as IL-5, IL-6 and IL-13 in response to IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) that are produced by epithelial cells. Group 3 ILC (ILC3s) including lymphoid tissue inducer (LTi) cells and LTi-like cells in adult produce IL-17 and IL-22 in response to IL-1β and IL-23. We focus on ILC2s that play important roles in helminth infection and allergic inflammation. We examined the regulatory mechanisms of ILC2 functions and demonstrate here that tissue-resident ILC2s proliferate *in situ* without migration during inflammatory responses. Both type I and type II IFNs, and interleukin-27 (IL-27) strongly suppressed proliferation and function of ILC2s in a STAT1 transcription factor-dependent manner. ILC2-mediated lung inflammation was enhanced in the absence of IFN-γ receptor on ILC2s *in vivo*, demonstrating the importance of endogenous IFN-γ in the termination of ILC2-mediated inflammation. IFN-γ effectively suppressed the function of ILC2s but not inflammatory ILC2s induced by IL-25, and IL-27 suppressed ILC2s but not T_H2 cells in *Alternaria*-induced lung inflammation. Our results demonstrate that IFN- and IL-27-mediated suppression is a negative feedback mechanism for ILC2 function *in vivo*.

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Follicular Helper T (Tfh) Cells Mediate IgE Antibody Responses to Airborne Allergens

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Background: Th2 cells have long been believed to play a pivotal role in regulating IgE antibody production. A new T cell subset, Tfh cells, is specialized in supporting B cell maturation and antibody production. The goal of this project was to investigate the roles of Th2 cells and Tfh cells in allergic immune responses by using mouse models.

Methods: Naïve mice were exposed intranasally to natural allergens or IL-1 family cytokines. Development of allergic immune responses was analyzed by collecting draining lymph nodes (LNs) and sera and by challenging mice with antigens. The roles of Tfh cells were examined by using conditional CD4-specific Bcl6-deficient mice.

Results: We found that Tfh cells and Th2 cells are developed when naïve mice are exposed through the airways to IL-1-family cytokines or natural airborne allergens, such as fungi and house dust mite. By adoptive transfer to mice deficient in T cells, Tfh cells supported long-lasting production of IgE antibodies to airborne antigens. Conditional deficiency of a master regulator Bcl6 in $CD4^+$ T cells (i.e. $Bcl6^{fl/fl}$*Cd4*-Cre mice) resulted in marked reduction in Tfh cell numbers and IgE antibody levels while these mice develop normal Th2-type immune responses in respiratory mucosa. When wild-type mice were exposed to peanut flour through the airways, they developed robust IgE and IgG antibodies to peanuts. Production of these antibodies and development of systemic anaphylactic response to peanuts were abolished in $Bcl6^{fl/fl}$*Cd4*-Cre mice.

Conclusions: These observations highlight a critical role for Tfh cells in mediating IgE antibody production to airborne allergens. Th2-type immune response and IgE antibody production are likely regulated by distinct sets of $CD4^+$ T cell subsets, namely Th2 cells and Tfh cells, respectively.

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CCL17 induces accumulation of myeloid dendritic cells (DC) and hyperresponsiveness in allergic airway inflammation

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Background: We previously showed that pulmonary dendritic cell (DC) maturation and migration was regulated by surfactant protein D (SP-D), an immunoprotective epithelial mediator. CCL17 is a proinflammatory chemokine ligand of CCR4, implicated in asthma. We found that CCL17 was expressed by DC in an autocrine manner and hypothesized that SP-D regulates this function. We studied the significance of CCL17 in O_3 and allergen-induced airway inflammation in mice.

Methods: The direct effects of SP-D on CCL17 expression were studied in bone-marrow derived (BM) DCs *in vitro*. C57BL/6, $SP\text{-}D^{-/-}$ and Balb/c mice were exposed to O_3 (3 ppm for 2hr) with or without prior sensitization/challenge with *Aspergillus fumigatus* (*Af*). Lung CCL17 protein and mRNA levels were determined. Cells from lung and bronchoalveolar lavage (BAL) were assessed for CCL17 and CCR4

expression by FACS. Cell migration was studied by PKH26 injection (i.v.). CCL17 blocking antibodies (150mg/mouse, i.p.) were given with the *Af* challenge and/or 12hr before O_3 exposure. Airway hyperesponsiveness was assessed by flexivent.

Results: CCL17 expression was increased during maturation of BMDCs and was attenuated in a time- and dose-dependent manner by recombinant SP-D *in vitro*. Genetically low SP-D expressor Balb/c mice showed heightened inflammation and greater CCL17 expression compared to C57BL/6 mice in response to O_3. After *Af* sensitization/challenge, O_3 enhanced eosinophilia and CCL17 and decreased SP-D expression in the airways of Balb/c mice. Consistently, SP-D$^{-/-}$ DCs had heightened CCL17 expression both at baseline and after *Af* and O_3 exposure in the airways. Expression of CCL17 in the airways was accompanied by increased accumulation of CD11b$^+$/CCR4$^+$/PKH26$^+$ DC and methacholine hyperresponsiveness post *Af* challenge that were further increased after O_3 exposure. Treatment with anti-CCL17 blocking antibodies significantly reduced airway resistance post O_3 exposure both in naïve and *Af* sensitized/challenged mice.

Conclusion: Thus, a heightened expression of CCL17 was associated with traffic of bone-marrow derived, phagocytic DC to the inflamed epithelium in response to O_3 or allergen exposure. Autocrine CCL17 was inhibited by SP-D. CCL17 blockade abolished central airway hyperresponsiveness but not inflammation in O_3- and allergen exposed mice indicating a novel direct effect of CCL17 on airway smooth muscle function.

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New Insights into T-Cell Immunity to Rhinovirus in Atopic Asthma.

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Background: Asthma exacerbations induced by rhinovirus (RV) infection exact an enormous toll on society. Atopy is a major risk factor for these episodes; however, the reasons are unclear. Current theories relate to deficient anti-viral immunity or else dysregulated Th2-driven inflammation. These viewpoints have been difficult to reconcile owing to the lack of in vivo models, and a dearth of experimental tools and technologies for analyzing T cells with any precision.

Method: We capitalized on the development of novel MHCII tetramers and state-of-the-art experimental platforms to construct a comprehensive profile of T-cell immunity to RV in the atopic host. For the first time, virus-specific and allergen-specific CD4+ T cells were tracked in parallel using multi-color flow cytometry in atopic asthmatics (n=10), following experimental inoculation with RV-16. Responses were compared to those in healthy non-atopic controls (n=10). T-cell transitions identified were further assessed in the context of cellular networks impacted by RV infection using mass cytometry and a 33-marker panel.

Results: Circulating RV- and allergen-specific memory T cells were readily identified prior to RV inoculation in atopic asthmatics using dual MHCII tetramer staining. Each T-cell population expanded during the effector phase of infection (7 days post-inoculation), but to differing degrees, thereby altering their relative contributions compared with baseline. Though discrete molecular signatures segregated according to T-cell specificity, these deviated from "classical" Th1 and Th2 phenotypes, shifted during infection, and were consistent with re-direction of circulating T cells to the respiratory tract. High-dimensional immunophenotyping of total circulating leukocytes by mass cytometry revealed synchronized emigration of a minor memory CD4+ T-cell population and memory B-cell subsets, which coincided with peak symptoms.

These T cells displayed a molecular signature akin to tetramer+ cells, and re-emerged 3 weeks after infection as an expanded population enriched for cells expressing the Th2-specific transcription factor, GATA-3. In healthy controls, allergen-specific T cells were rare and not modulated during RV infection.

Conclusions: Our findings confirm that the T-cell response to RV in atopic asthma comprises a mixture of virus- and allergen-specific cells with heterogeneous phenotypes. We propose that co-opting of allergen-specific T cells subverts Th1 immunity in infected asthmatics.

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Ovarian hormones increase ILC2 cytokine expression and Alternaria extract-induced airway inflammation

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Background: There is a gender disparity associated with asthma that changes throughout life. Around puberty there is a shift in asthma prevalence, and as adults women are twice as likely as men to have asthma. Group 2 innate lymphoid cells (ILC2) are a subgroup of ILCs that produce IL-5 and IL-13, cytokines critical for increased airway inflammation associated with asthma. Currently, the role of sex hormones on ILC2 mediated airway inflammation remains unknown. We hypothesized that ovarian hormones increase ILC2 cytokine expression and ILC2-induced airway inflammation.

Methods and Results: To test our hypothesis, we first isolated ILC2 from the lungs of female and male BALB/c mice, and stimulated the ILC2 *ex vivo* with IL-2 (10ng/ml) and IL-33 (10ng/ml) for 6 days. IL-5 and IL-13 protein expression, measured by ELISA from culture supernatants, was significantly increased in lung ILC2 from female mice compared to male mice. Next, we intranasally administered an extract of the *Alternaria alternata* (Alt Ext) for 4 consecutive days to sham-operated or gonadectomized female and male BALB/c mice (n=8 mice per group, $p<0.05$ significant). Alt Ext is an aeroallergen associated with asthma exacerbations that we have previously shown induced lung ILC2 production of IL-5 and IL-13. Twenty-four hours after the last Alt Ext challenge, bronchoalveolar lavage (BAL) fluid and lungs were harvested. Alt Ext induced a significant increase in IL-5, IL-13, and IL-33 protein expression in the BAL fluid of sham-operated female mice compared to the BAL fluid of sham-operated male and gonadectomized female and male mice. Total lung ILC2 cells as well as IL-5+ and IL-13+ ILC2 were also significantly increased in Alt Ext treated sham-operated female mice compared to Alt Ext sham-operated male mice and gonadectomized female or male mice. Further, infiltration of eosinophils and lymphocytes was increased in Alt Ext treated sham-operated female mice compared to gonadectomized female and male mice and sham-operated male mice.

Conclusions: Our results show that ovarian hormones increase lung ILC2 cytokine expression and provide a potential mechanism for increased asthma prevalence in women compared to men.

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Increase in Activated Group 2 Innate Lymphoid Cells in the Airway of Mild Asthmatics Following Allergen Inhalation Challenge

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Background: Group 2 innate lymphoid cells (ILC2), are a novel cell type that are lineage-negative, lack antigen specificity and are a major source of type 2 cytokines (IL-5 and IL-13). In murine models of asthma, ILC2 facilitate allergen-driven eosinophilic inflammatory responses in the absence of CD4+ T lymphocytes although both cell types have been shown to co-operate for a maximal inflammatory responses in the lung. We have shown that increased localized activation of ILC2 is associated with uncontrolled airway eosinophilia in prednisone-dependent severe asthmatics. Here we investigated the kinetics of lung-homing and activation of ILC2 and CD4 lymphocytes following allergen-inhalation challenge with a view to understanding the relative contribution of these cells to the development of airway eosinophilia and lung dysfunction in mild asthma.

Methods: In a diluent-controlled allergen (Ag)-challenge cross-over study, ILC2s (lin-FcεRI-CD45+CD127+ST2+), CD3+CD4+ T lymphocytes and intracellular IL-5 and IL-13 expression were enumerated by flow cytometry in steroid-naive mild atopic asthmatics. All subjects (n=9) developed Ag-induced dual bronchoconstrictor response, airway eosinophilia and delayed methacholine airway hyperresponsiveness. Bone marrow aspirates, blood and sputum samples were collected pre-, 24h and 48h post-challenge and immediately fixed in 1% paraformaldehyde prior to immunofluorescence staining.

Results: Compared to pre-Ag levels, there was a rapid and discreet increase in sputum levels of ILC2 at 24h ($P<0.01$) which decreased to baseline levels 48h post-Ag. This was co-incident with a significant decrease in blood and bone marrow ILC2s at 24h post-Ag ($P<0.05$). In sputum, the number of activated ILC2 (IL5+ and IL13+) increased significantly 24 h post-Ag compared to pre-Ag while no change in activated ILC2 cells was detected in the blood or bone marrow post-Ag. Total CD4+ T cells and IL-13+CD4+ T cells increased at 24h post-Ag ($P<0.01$) and remained elevated at 48h post-Ag ($P<0.05$) while IL-5+CD4+ T cells only showed a trend for an increase at 48h post-Ag in sputum.

Conclusions: Our findings suggest that following allergen challenge localized activation of ILC2 within the airways may initiate the development of eosinophilic inflammation in mild asthmatics while type 2 cytokines produced by CD4+ T lymphocytes may be involved in the persistence of the asthmatic response.

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Deadline for Submission: 21 October 2016

31st Symposium of the

Collegium Internationale Allergologicum

3–8 APRIL 2016

Charleston, South Carolina

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