29th Symposium of the Collegium Internationale Allergologicum

14 – 19 October 2012

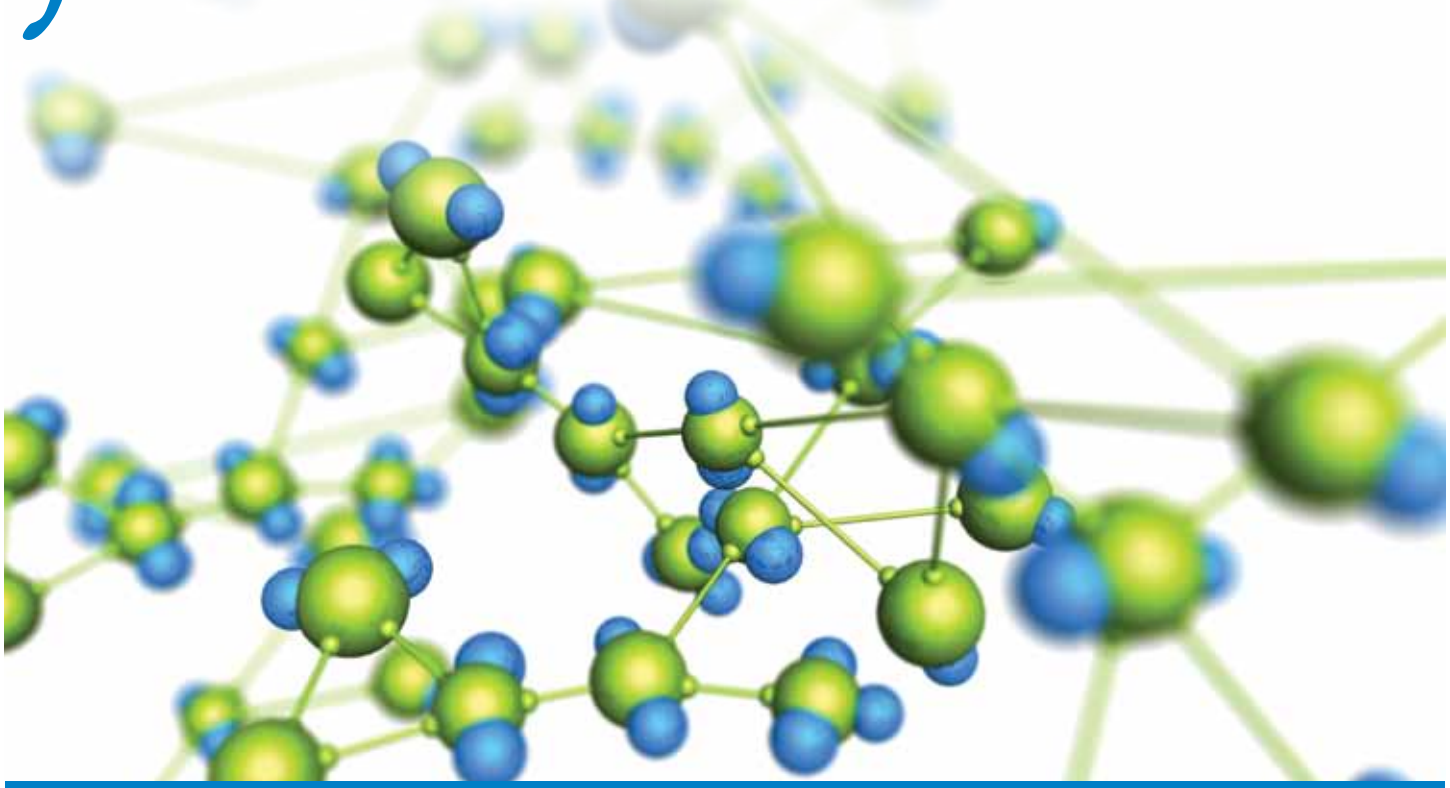
COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

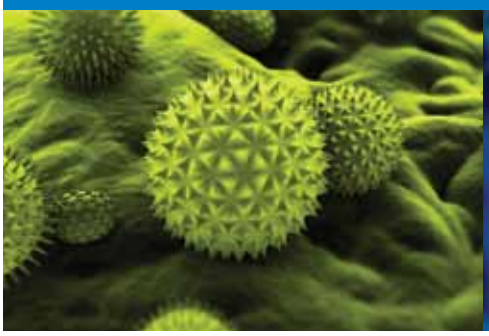
Jeju Island

Republic of Korea

Allergic Diseases: from Mechanisms to Cures



Final Program



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29th Symposium of the Collegium Internationale Allergologicum

Jeju Island
Republic of Korea

14 – 19 October 2012

Dear Colleagues,

It is our great pleasure to welcome you to the 29th Symposium of the Collegium Internationale Allergologicum entitled ***Allergic Diseases: from Mechanisms to Cures*** from **14-19 October 2012** in **Jeju Island, Republic of Korea**.

The purpose of this meeting, like all meetings of the *Collegium*, is to bring basic and translational scientists, clinical immunologists, and allergists together to foster interdisciplinary approaches to understand, treat, and, ultimately, cure immunological disorders.

As in previous meetings, the majority of the scientific program will be given over to free communications that will be presented either orally or as poster discussions. The *Collegium's* unique organizational structure and membership guidelines ensure that the science presented at the biennial symposia of the *Collegium* is not only important, but includes many late-breaking and in many cases unpublished developments.

We believe you will find that the venue selected by the Council of the *Collegium* for its 29th meeting will accommodate scientists and accompanying persons in the tradition and style that have characterized past meetings of the *Collegium*.

Jeju Island is a volcanic island, dominated by Halla-san (Halla Mountain): a volcano 1,950 meters high and the highest mountain in South Korea. The weather on Jeju typically is pleasantly mild in October, which is widely considered to be the best time of the year to visit. Because of the relative isolation of the island, the people of Jeju have developed a culture and traditions that are distinct from those of mainland Korea. In addition, those who have a chance to stray away from the meeting site will find that Jeju is full of breathtaking sights and unusual attractions, including three UNESCO World Natural Heritage sites, which highlight the island's magnificent combination of natural beauty, diverse ecosystems, and striking geological formations. In recognition of its extraordinary natural features, Jeju has been named one of the New 7 Wonders of Nature in 2011.

On behalf of the *Collegium* Council Members, and the group of local organizers who have worked so effectively to prepare for this meeting, thank you for attending the 29th Symposium on Jeju Island. From the beginning, the *Collegium* was intended to be an exclusive group of friends pursuing excellence in research in a spirit of open intellectual exchange at meetings held in interesting and stimulating locations. We will again strive to fulfill this vision over the next week in Jeju.

Yours sincerely,

Stephen J. Galli, MD
President and Symposium Organizer

You-Young Kim, MD, PhD
Symposium Organizer

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General Information

The 29th Symposium of the *Collegium* will be held on beautiful Jeju Island, recently named one of the New 7 Wonders of Nature, off the coast of the Republic of Korea. Jeju Island is a volcanic island, dominated by Halla Mountain, the highest mountain in South Korea. The island measures approximately 109 miles across, end to end, at the widest points. The island was created entirely from volcanic eruptions and consists chiefly of basalt and lava. It has a humid subtropical climate, warmer than that of the rest of Korea, with four distinct seasons. Winters are cool and dry while summers are hot, humid, and sometimes rainy.

Airport Transfers

Transfers to and from the Jeju Island Airport and the Incheon/ Gimpo airports are included in the registration fee. *Please confirm your departure pick-up time at the Registration Desk located in the Shilla Jeju Hotel.*

CME

The 29th Symposium of the Collegium Internationale Allergologicum will offer 22 Continuing Medical Education (CME) credits through the European Accreditation Council for Continuing Medical Education (EACCME). EACCME credits are recognized by the American Medical Association (AMA) toward the Physician's Recognition Award (PRA). To convert EACCME to AMA PRA category 1 credit, contact the AMA at 800 262 3211.

In order to receive your CME certificate by email, please fill out the CME Self-Reporter Form located in your registration packet and return it to the Registration Desk in the Shilla Jeju Hotel.

Coffee Breaks

Coffee breaks are included in the registration fee for delegates and will be served daily. Coffee will be served outside of Halla Hall; please check the Schedule of Events for exact times and locations.

Currency

The currency used on Jeju Island is the South Korean Won (KRW). There are ATMs, or Bancomats, widely available for cash withdrawal. Credit cards are also accepted at most hotels, restaurants and shops.

Electricity

The electrical current in the Republic of Korea is AC - 220-240 Volts. The European adapter with round prongs is acceptable.

Evaluations

Evaluation forms are included in the registration packet. Please fill out your form and return it to the Registration Desk in the Shilla Jeju Hotel. You must fill out your evaluation in order to receive your CME certificate.

Hospitality Desk

The Hospitality Desk is located next to the Registration Desk at the Shilla Jeju Hotel. Delegates and accompanying persons will be able to sign up for excursions and ask for advice regarding activities on Jeju Island.

Hours:

Monday, 15 October	8:00 – 12:00
Wednesday, 17 October	7:00 – 11:00
Thursday, 18 October	7:00 – 11:00

Language

The official language of the 29th Symposium is English, and the official language of Jeju Island is Korean.

"Life in Science" Breakfast Discussions

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

Space is limited; please sign up at the registration desk to attend these sessions.

Lunches

Buffet-style lunches will be served in the Shilla Jeju Hotel Parkview restaurant during the following times:

Monday, 15 October	13:00 – 14:30
Tuesday, 16 October	11:30 – 12:30
Wednesday, 17 October	13:30 – 15:00
Thursday, 18 October	13:30 – 15:00
Friday, 19 October	12:30 – 13:30 (ticket purchase required)

Lunch is included in the registration fee for delegates, accompanying persons and children (except Friday, 19 October.)

Oral Abstract Sessions

Oral Abstract Sessions will take place on 15, 16, 17, 18, and 19 October. All Oral Abstract Sessions will take place in the Shilla Jeju Hotel.

All Oral Abstract Session presenters will be given 12 minutes to speak and 5 minutes for question and answer. It is the responsibility of the chairperson to keep the session on time.

Poster Sessions

Poster Sessions will take place from 17:00 – 19:00 on 15, 17, and 18 October in Lotus Hall of the Shilla Jeju Hotel. An assortment of wine, cheese and other refreshments will be served. Poster presenters will stand next to their posters during the session and be available for questions and discussion.

Proceedings

Papers from the 29th Symposium will be published by Pacini Editore.

Online submission is now open at http://cia.execinc.com/edibo/Abstracts

Deadline for Submission: 30 March 2013

General Information

Registration

The Registration Desk is located in the Shilla Jeju Hotel on all days of the Symposium.

Hours:

Sunday, 14 October	14:00 – 20:00
Monday, 15 October	6:30 – 13:30
	15:00 – 19:00
Tuesday, 16 October	7:30 – 12:00
Wednesday, 17 October	7:30 – 13:30
	15:00 – 19:00
Thursday, 18 October	7:30 – 13:30
	16:00 – 20:00
Friday, 19 October	7:30 – 13:00

Registration Fees

The registration fee for delegates includes:

Airport Transfer on Arrival

- From Incheon airport in Seoul to Gimpo airport in Seoul for connecting flights
- From the airport on Jeju Island to the hotels

Airport Transfer on Departure

- From the hotels to the airport on Jeju Island
- From Gimpo airport in Seoul to Incheon airport in Seoul

Oral Abstract Sessions

Poster Sessions

Coffee Breaks

Lunches

Social Events

The registration fee for accompanying persons and children includes:

Airport Transfer on Arrival

- From Incheon airport in Seoul to Gimpo airport in Seoul for connecting flights
- From the airport on Jeju Island to the hotels

Airport Transfer on Departure

- From the hotels to the airport on Jeju Island
- From Gimpo airport in Seoul to Incheon airport in Seoul

Lunches

Social Events

Speaker Preview Room

The Speaker Preview Room is located in the Shilla Jeju Hotel's Mara Room. Speakers will be able to check and upload their presentations before the Oral Abstract Sessions.

Hours:

Monday, 15 October	6:30 – 17:00
Tuesday, 16 October	7:30 – 12:00
Wednesday, 17 October	7:30 – 17:00
Thursday, 18 October	7:30 – 15:00
Friday, 19 October	7:30 – 12:30

Time Zone

Jeju Island is on South Korea Time Standard, which is nine hours ahead of Greenwich Mean Time (GMT).

Tipping

Tipping is not required nor expected in Korea, but most major hotels add a compulsory 10% service charge to bills. This is on top of the 10% VAT (which is usually included in prices at most stores in Korea, but not in some high-end restaurants). Taxi drivers will appreciate it if you tell them to "keep the change," but this is not expected.

Venue

The scientific program of the 29th Symposium will take place in the Shilla Jeju Hotel.

Social Events

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

Welcome Reception

Sunday, 14 October 2012, 18:30 – 21:30

The Welcome Reception will be held at the Shilla Jeju Hotel Gardens. Musical entertainment will be provided along with refreshments and an assortment of hors d'oeuvres.

Spirited Garden and Boat Ride

Tuesday, 16 October 2012, 13:30 – 18:30

In the afternoon, attendees will visit the Spirited Gardens which has been recognized as one of the most beautiful bonsai gardens in the world. Following in the tradition of past *Collegium* meetings, a boat ride will take place on the third day of the meeting. Participants will enjoy a leisurely cruise with fantastic views of the amazing natural formations along the southern perimeter of Jeju Island.

Informal Dinner

Tuesday, 16 October 2012, 20:00 – 22:30

The Informal Dinner will take place at the Jeju International Convention Center. The evening will include a performance by NANTA, a popular non-verbal performance that dramatizes Korean percussion in a comedic stage show.

Gala Dinner

Thursday, 18 October 2012, 19:30 – 23:00

An elegant dinner will be held on the last evening of the Symposium at the Shilla Jeju Hotel. Entertainment for the evening will include a special nature-themed fashion show organized in conjunction with the Duksung Women's University, a vocal performance given by the Seoul National University of Music and music with a chance for participants to engage in light ballroom dancing. This will be a semi-formal event and all men are requested to wear bow-ties. Those who wish to wear evening clothes (i.e., tuxedos) may do so, but that is optional. Complimentary silk bow-ties or scarves will be given as gifts to all participants.

Optional Excursions

All tours will depart from the lobby of the Shilla Jeju Hotel, at the start times listed below.

To register for a tour, please visit the Hospitality Desk, located in the Shilla Jeju Hotel.

Halla Mountain

Sunday, 14 October 2012

8:30 – 15:30

Halla Mountain is the central peak of Jeju Island, a shield volcano formed from the continental shelf of the Yellow Sea and, at 1,950 meters, one of the tallest mountains in Korea. Halla Mountain boasts spectacular and wondrous landscape as a result of its varied volcanic topography and vertical ecosystem of plants capped at its peak by the crater lake Baengnokdam. Participants will ascend Halla Mountain via the Yeongsil Trail, which consists of a 2.4 km road from the Yeongsil Control Station (altitude 1,000 m) to the Yeongsil Rest Area (altitude 1,280 m), and a 3.7 km foot trail from the Yeongsil Rest Area to the Witsae-oreum Shelter (altitude 1,700 m). A one-way trip to Witsae-oreum Shelter generally takes a little over two hours. With the exception of the somewhat steep Yeongsil Crater Ridge (altitude 1,300 – 1550 m), most of the trail is very even and easy to hike.

Price per person: €45

Tour fee includes transportation to/from Halla Mountain, English speaking guide and packed lunches.

Cheonjiyeon Waterfall / Seogwipo Submarine*

Monday, 15 October 2012

13:50 – 18:00

Cheonjiyeon, meaning "God's pond," derives its name from the legend that the seven fairies serving the King of Heaven came down to the pond on stairs of cloud and bathed in its clean waters. The name Cheonjiyeon literally means sky (Ch'eon) connected with land (ji). In order to reach the waterfall, visitors must walk along a landscaped trail whose entrance is marked by a traditional Korean raft called a T'e-u.

The Seogwipo Submarine descends to a depth of about 40 meters and allows passengers to safely explore the undersea world beneath the surface of Jeju Island. The ride lasts approximately 65 minutes during which passengers will encounter many forms of marine plant and animal life and even a wreckage. *The submarine ride is optional and tickets are an additional cost.

The Jusangjeolli are stone columns which line the southern coast and are a designated natural monument of Jeju Island. The Jusangjeolli were formed when lava from Halla Mountain flowed into the sea of Jungmun. These volcanic pillars are shaped like cubes or hexagons of various sizes and almost seem as if they were handmade by stonemasons.

Price per person: €60
€94 (includes optional submarine ride)

Tour fee includes transportation, English speaking guide and admission fees.

Sangumburi Crater / Manjang Cave

Thursday, 18 October 2012

8:00 – 12:00

Sangumburi is perhaps the most impressive of Jeju's many volcanic craters. This particular type is known as a Marr crater, as it was produced by an explosion in a generally flat area. A short climb to the top affords sweeping views of pristine Jeju terrain. Due to the abundance of unique and exotic plant life that grows in the crater, it has attracted the attention of many researchers as well.

The 7,416-meter long Manjang Cave has officially been recognized as the longest lava tube in the world. Extremely well-preserved lava stalactites, stalagmites, columns, flowstone, cave coral, benches, lava rafts, bridges, shelves, and grooved lava striations can be found in the cave. Among these, a 7.6 meter lava column is the largest known in the world. The Geomunoreum Lava Tube system, of which Manjang Cave is a part, was designated a UNESCO World Natural Heritage site in 2007.

Price per person: €45

Tour fee includes transportation, English speaking guide and admission fees.

Alain L. de Weck Travel Grant Recipients

For the second 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:

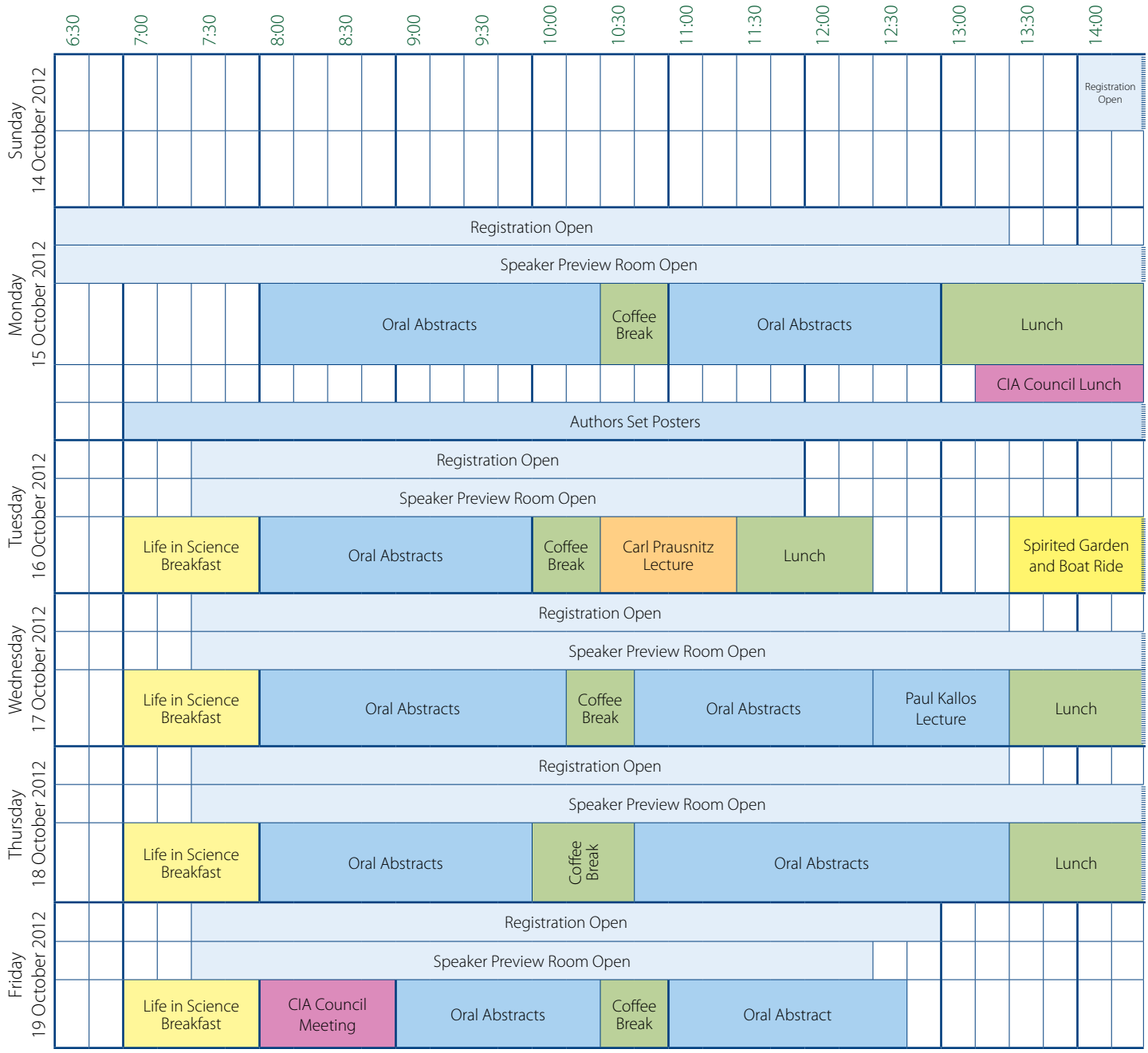
David Cousins, United Kingdom
Michele Grimbaldeston, Australia
Jan Gutermuth, Germany
Tillie-Louise Hackett, Canada
Hans Michael Haitchi, United Kingdom
Carl Hamsten, Sweden
Xingnan Li, United States
Katharina Marth, Austria
Tae Chul Moon, Canada
Kari Nadeau, United States
Taiji Nakano, Japan
Kanami Orihara, Canada
Pia-Lauren Reece, Canada
Mohamed Shamji, United Kingdom
Emily Swindle, United Kingdom
Kazuto Taniguchi, Japan
Stephen Till, United Kingdom

Travel Grant Recipients will be awarded with a certificate during the Gala Dinner on 18 October 2012.

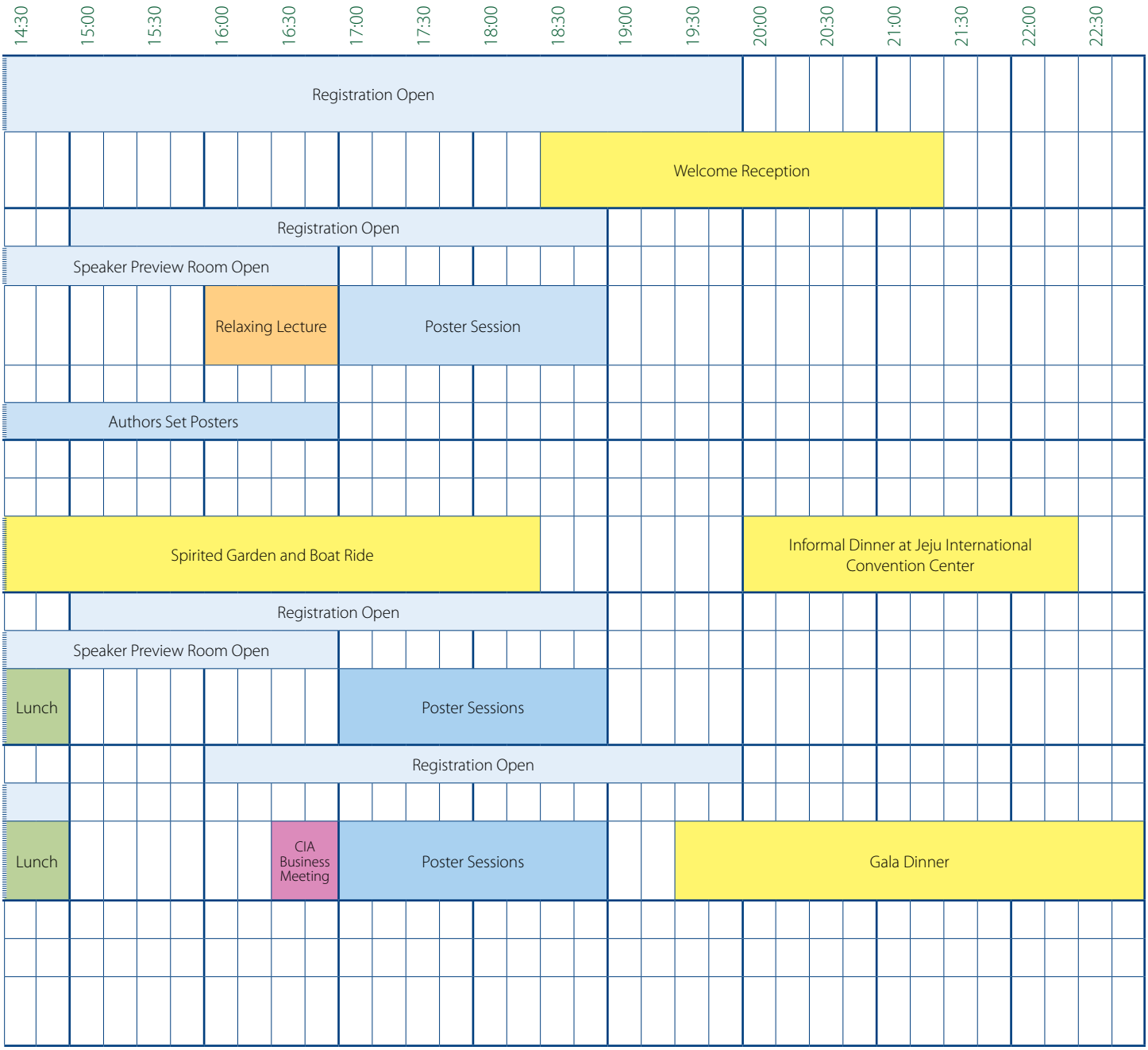
The *Collegium* would like to thank the members who contributed to the Alain L. de Weck Travel Grant Fund (included in the membership renewal form) and the following company:

INDOOR biotechnologies

Program-at-a-Glance



Program-at-a-Glance



Map of Hotels

Schedule of Events

Sunday, 14 October 2012

14:00 – 20:00 Registration Opens ..... Shilla Jeju Hotel

18:30 – 21:30 Welcome Reception ..... Shilla Jeju Hotel
Stephen J. Galli, United States
You-Young Kim, Korea

Monday, 15 October 2012

6:30 – 13:30 Registration Open ..... Shilla Jeju Hotel

6:30 – 17:00 Speaker Preview Room Open ..... Ora Room

7:00 – 17:00 Authors Set Posters ..... Lotus Hall

8:00 – 10:35 Oral Abstract Session 1 ..... Halla Hall
Genetic and Environmental Factors in Allergic Disorders
Chairpersons: You-Young Kim, Korea
Donata Vercelli, United States

8:00 1 *Molecular genetics of atopic eczema*
Johannes Ring, Germany

8:17 2 *Asthma phenotypes: Heterogeneity and genomics*
Eugene Bleecker, United States

8:34 3 *Asthma associated variants in IL-13 upregulate human IL-4 expression in mice humanized for the Th2 cytokine locus*
Donata Vercelli, United States

8:51 4 *Genome-wide association studies of total serum IgE levels in asthma*
Xingnan Li, United States

9:08 5 *Role of genetics in severe asthma*
Deborah Meyers, United States

9:25 6 *Genome wide association study of aspirin-intolerant asthma in the Japanese population*
Mayumi Tamari, Japan

9:42 7 *Molecular genetic mechanisms of eosinophil activation in patients with ASA exacerbated respiratory disease*
Hae-Sim Park, Korea

9:59 8 *Climate change, pollen and allergy*
Heidrun Behrendt, Germany

10:16 9 *Metagenomics of microbiota and extracellular vesicles in indoor dust and stools*
Yoon-Keun Kim, Korea

10:35 – 11:00 Coffee Break ..... Halla Hall Lobby

11:00 – 13:00 Oral Abstract Session 2 ..... Halla Hall
Lymphocytes and Mediators of Immunoregulation
Chairpersons: Mubeccel Akdis, Switzerland
John R. Gordon, Canada

11:00 10 *Amphiregulin, a potential survival factor, is produced by $CD4^+$ T cells following NMDA receptor activation*
Kanami Orihara, Canada

11:17 11 *Induced regulatory T cells are distinctly superior to natural Treg of the same allergen specificity in their abilities to induce asthma tolerance*
John R. Gordon, Canada

11:34 12 *Induction of Foxp3+ regulatory T cells by Lactobacillus rhamnosus: A critical role for heme oxygenase 1*
Paul Forsythe, Canada

11:51 13 *IL-35+CD4+CD25+Foxp3- regulatory T cells suppress Th2 immune responses and are induced following grass pollen-specific sublingual immunotherapy*
Mohamed H. Shamji, United Kingdom

12:08 14 *In vitro differentiation and transcriptome profiling of human Th9 cells*
David J. Cousins, United Kingdom

Monday, 15 October 2012 (continued)

12:25 15 *Human B regulatory 1 cells suppress antigen-specific immune responses and develop IgG4-producing plasma cells*
Mubeccel Akdis, Switzerland

12:42 16 *Role of natural helper cells in the lung inflammation*
Shigeo Koyasu, Japan

13:00 – 14:30 Lunch ..... Shilla Jeju Hotel

13:15 – 14:30 CIA Council Meeting ..... Lily Room

15:00 – 19:00 Registration Open ..... Shilla Jeju Hotel

16:00 – 17:00 Relaxing from Immunology (accompanying persons included) ..... Halla Hall
The Dark Lady of DNA
Chairperson: Hannah Gould, United Kingdom
Brenda Maddox

Brenda Maddox was born in Brockton, Massachusetts, she attended Bridgewater High School and then graduated from Harvard University, class of 1953, with an honors degree in English literature. After becoming a journalist on the Quincy, MA *Patriot-Ledger*, she moved to London in 1959 to study at the London School of Economics, and then moved to work at *Reuters* and later *The Economist* where she was on the staff for many years, latterly as Britain editor. She has been a book reviewer for publications on both sides of the Atlantic, including *The Observer*, the Times Literary Supplement and *The New York Times* and a frequent broadcaster on BBC Radio 4. She has written many biographies, including one on D.H. Lawrence, which won the Whitbread Biography Prize in 1988. Her lives of Nora Joyce, W.B. Yeats and Rosalind Franklin have been widely acclaimed. She has won the Los Angeles Times Biography Award, the Silver PEN Award, the French Prix du Meilleur Liver Étrange, and the Whitbread Biography Prize.

She was married for 49 years to the acclaimed editor of *Nature*, the late Sir John Maddox, (who died in 2009) and with him, a native of Wales, bought an eighteenth century farmhouse in mid-Wales, which remains a place for the gathering of her family which includes Bronwen Maddox, editor of Prospect magazine, Bruno Maddox, columnist and novelist, and her granddaughter Laura.

17:00 – 19:00 Poster Session 1 ..... Lotus Room
Genetic and Environmental Factors in Allergic Disorders
Chairpersons: Heidrun Behrendt, Germany
Deborah A. Meyers, United States

17 *Expression of hsa-mir-15a, which is a candidate microRNA regulating VEGFA expression, is lower in CD4⁺ T cells in pediatric asthma patients*
Taiji Nakano, Japan

18 *Reduced diversity within the gut Bacteroidetes phylum in early infancy associates with delivery by caesarean section, atopic eczema development and delayed Th1 maturation*
Maria Jenmalm, Sweden

19 *Gene-environment interaction between TLR4/CD14/IL-13 polymorphism and bronchiolitis may influence the development of asthma in children*
Soo-Jong Hong, Korea

20 *The evolution of IgE sensitization to Ascaris allergenic components in early infancy*
Luis Caraballo, Columbia

21 *Bacteria-derived extracellular vesicles as an important causative agent for asthma and COPD*
Young-Koo Jee, Korea

22 *Utilization of nasal lavage exosomes as biomarkers in asthma with and without chronic rhinosinusitis – a proteomics approach*
Jan Lötvall, Sweden

23 *Silica crystals cause cellular damage in TLR3-activated human bronchial epithelial cells*
Akio Matsuda, Japan

24 *The burden of asthma and perceptions of asthma control In South Korea: Results of the Asia-Pacific (AP) countries asthma insight and management (AIM) survey of patients*
Young Joo Cho, Korea

Monday, 15 October 2012 (continued)

25 *Prevalence of IgE-binding to Art v, Amb a and Hum s among pollen skin test positive patients from Northern China*
Michael D. Spangfort, Hong Kong

26 *The development of atopic dermatitis according to age of onset and the association with early life exposures*
Caroline Roduit, Switzerland

17:00 – 19:00 Poster Session 2 ..... Lotus Room
Lymphocytes and Mediators of Immunoregulation
Chairpersons: David M. Kemeny, Singapore
Shigeo Koyasu, Japan

28 *Differential impact of glutamate and kynurenines on CD4+ T cell subtype function*
Kanami Orihara, Canada

29 *How splenic long-lived plasma cells impinge on humoral immunity*
Jeehee Youn, Korea

30 *CD8 T cells: master regulators of the immune response in infection and inflammation*
David M. Kemeny, Singapore

31 *Development of the innate immunity in childhood and the association with environmental exposures*
Caroline Roduit, Switzerland

32 *Contact dermatitis in psoriasis patients: A powerful model to analyze disease pathogenesis*
Kilian Eyerich, Germany

33 *Phospholipase A_2 of peroxiredoxin 6 has a critical role in tumor necrosis factor-induced apoptosis in human bronchial epithelial cells (BEAS2B)*
Ki-Young Lee, Korea

34 *Characterisation of grass pollen allergen-driven memory B cells in human patients with allergic respiratory disease*
Janet Davies, Australia

17:00 – 19:00 Poster Session 3 ..... Lotus Room
Pathophysiology of Allergic Disorders and Inflammation
Chairpersons: Christopher J. Corrigan, United Kingdom
Hee-Bom Moon, Korea

35 *Bronchial mucosal leukocytes in aspirin sensitive asthma show deficient expression of the PGE2 receptor EP2*
Christopher J. Corrigan, United Kingdom

36 *Human milk oligosaccharides reduce respiratory viral infection and inflammation in human cells*
Rachael H. Buck, United States

37 *Airway immunoexpression of CD206, a marker of alternatively activated macrophages, is increased in asthma and influenced by disease severity and inhalation allergen challenge*
Nicholas Dragolea, United Kingdom

38 *Chronic chlorine exposure aggravates allergic asthma via innate immune system in a murine model of asthma*
You-Young Kim, Korea

39 *Phytoncide inhibits asthmatic reaction in murine asthma model*
Mi-Kyeong Kim, Korea

40 *Allergic airway inflammation and airway remodeling are more severe in female mice*
Masamichi Itoga, Japan

41 *Effect of NADPH oxidase 2 (NOX2) deficiency on allergic lung inflammation and differentiation of helper T cells*
Seung-Hyo Lee, Korea

42 *Molecular allergy diagnostics in allergic rhinoconjunctivitis to grass pollen – A prerequisite for component-resolved specific immunotherapy*
Johannes Huss-Marp, Germany

43 *Long-term exposure to IFN-gamma enhances Th2 cytokine-induced gene expression in conjunctival fibroblast*
Naoko Okada, Japan

44 *Allergen-specific T and B cell proliferation determined in pollen allergic patients by a CFSE dilution based assay*
Julia Eckl-Dorna, Austria

Monday, 15 October 2012 (continued)

45 *Antigen-specific Th2-type immune responses certainly underlie the pathogenesis of non-IgE-mediated gastrointestinal food allergy*
Hideaki Morita, Japan

46 *The prevalence of gastrointestinal reflux disease in chronic unexplained cough*
Byung-Jae Lee, Korea

47 *The IL-1alpha/periostin/IL-6 axis contributes to the keratinocyte proliferation and differentiation in atopic dermatitis*
Kazuto Taniguchi, Japan

48 *Host-pathogen interactions via bacteria-derived extracellular vesicles*
Yong Song Gho, Korea

49 *Vitamin D levels and allergic diseases in Korean adults: The 2010 Korean National Health and Nutrition Examination Survey*
Ho Joo Yoon, Korea

50 *Investigation of the mechanisms of delay in anaphylactic and urticarial responses to red meat in patients with IgE antibodies to alpha-gal*
Thomas A.E. Platts-Mills, United States

Tuesday, 16 October 2012

7:00 – 8:00 *"Life in Science" Breakfast Discussion* ..... Rose Room
Donata Vercelli, United States

7:30 – 12:00 Registration Open ..... Shilla Jeju Hotel

7:30 – 12:00 Speaker Preview Room Open ..... Ora Room

8:00 – 9:45 Oral Abstract Session 3 ..... Halla Hall
Pathophysiologic and Clinical Features of Allergic Disorders
Chairpersons: Cezmi Akdis, Switzerland
F. Estelle R. Simons, Canada

8:00 51 *The impact of allergenic substances on epithelial barrier function in severe asthma*
Donna Davies, United Kingdom

8:17 52 *Role of bronchial epithelial cells as a source of TF production and regulator of angiogenesis in asthma*
Jeffrey M. Drazen, United States

8:34 53 *Distinct effects of Th1, Th2, Th17 and Treg subsets on regulation of bronchial epithelial tight junctions*
Cezmi Akdis, Switzerland

8:51 54 *The role of caveolin-1 in epithelial barrier dysfunction in asthma*
Tillie-Louise Hackett, Canada

9:08 55 *A novel aspect of autoallergy: Dichotomic IgE-autoreactivity- pattern of skin/gut versus airway epithelial cells*
Jan Gutermuth, Germany

9:25 56 *A role for CCR3-CCL28 in T cell homing to the upper airway mucosa*
Frode Jahnsen, Norway

9:45 109 *Evidence for functional cross-talk between TLR2 and the high affinity receptor for IgE (FcERI) on human Langerhans cells*
Thomas Bieber, Germany

10:02 – 10:30 Coffee Break ..... Halla Hall Lobby

Tuesday, 16 October 2012 (continued)

10:30 – 11:30 **Carl Prausnitz Lecture** ..... **Halla Hall**

Vascular Permeability: Why is it Important?

Chairperson: Stephen J. Galli, United States

Harold Dvorak, MD
Mallinckrodt Distinguished Professor of Pathology
Harvard Medical School
United States

Dr. Harold F. Dvorak is an internationally recognized physician-scientist. He has published more than 200 original contributions and nearly 100 reviews. He is best known for his discovery of vascular permeability factor (VPF, now more generally known as VEGF-A), and, more recently, for his demonstration of the heterogeneity of the tumor vasculature, his characterization of the several different vessel types that comprise that vasculature, and their differential sensitivity to anti-VEGF therapy. He received undergraduate training at Princeton University and his medical degree from Harvard Medical School with honors in 1963. He did internship and residency training in Pathology at the Massachusetts General Hospital (MGH), spent two years as a research fellow at the National Institutes of Health, and returned to a faculty position at the MGH, rising to the rank of Professor of Pathology at Harvard in 1977. In 1979 Dr. Dvorak was called to chair the Department of Pathology at the then Beth Israel Hospital (now Beth Israel Deaconess Medical Center (BIDMC)) as Mallinckrodt Professor of Pathology. He held that position with distinction for 26 years, building a department that is now widely admired for both clinical and research excellence. He stepped down from the chairmanship in 2005 to return to the lab full time as Mallinckrodt Distinguished Professor of Pathology. In the same year he founded the Center for Vascular Biology at BIDMC.

11:30 – 12:30 Lunch ..... Shilla Jeju Hotel

13:30 – 18:30 Boat Ride and Spirited Garden Tour ..... Departs from Shilla Jeju Hotel Lobby

20:00 – 22:30 Informal Dinner ..... Jeju International Convention Center

Wednesday, 17 October 2012

7:00 – 8:00 *"Life in Science" Breakfast Discussion* ..... Rose Room
Stephen J. Galli, United States

7:30 – 13:30 Registration Open ..... Shilla Jeju Hotel

7:30 – 17:00 Speaker Preview Room Open ..... Ora Room

8:00 – 10:20 **Oral Abstract Session 4** ..... **Halla Hall**

Pathophysiologic and Clinical Features of Allergic Disorders

Chairpersons: Bruce S. Bochner, United States
Claudia Traidl-Hoffmann, Germany

8:00 57 *Characterization of allergen-specific T cell responses in humanized allergy mice*
Winfried Pickl, Austria

8:17 58 *Effects of a new potent poly (ADP rose) polymerase inhibitor in guinea pig allergic asthma*
Emanuela Masini, Italy

8:34 59 *Bitter taste receptors (TAS2Rs) are expressed on leukocytes from asthmatics and upregulated in children with severe asthma*
Sven-Erik Dahlén, Sweden

8:51 60 *Active soluble ADAM33 in BAL fluid is associated with airway remodeling and bronchial hyperresponsiveness in allergic airway inflammation mouse models*
Hans Michael Haitchi, United Kingdom

9:08 61 *From metabolome to function: Low molecular weight factors from pollen are modulators and aggravators of the allergic immune response in vitro and in vivo*
Claudia Traidl-Hoffmann, Germany

9:25 62 *Histamine-releasing factor (HRF) in asthma and atopic dermatitis*
Toshiaki Kawakami, United States

Wednesday, 17 October 2012 (continued)

9:42 63 *Histamine receptor 2 regulates respiratory allergy and inflammation*
Liam O'Mahony, Switzerland

9:59 64 *Role of IL-33 in allergy*
Susumu Nakae, Japan
2011 PhARF Award Winner Sponsored by Thermo Fisher Scientific
Introduction by Stephen J. Galli and remarks by Ruby Pawankar

10:20 – 10:45 Coffee ..... Halla Hall Lobby

10:45 – 12:30 Oral Abstract Session 5 ..... Halla Hall
Pathophysiologic and Clinical Features of Allergic Disorders
Chairpersons: Philippe Gevaert, Belgium
Yoseph Mekori, Israel

10:45 65 *Local polyclonal IgE in nasal polyps with asthma is functional*
Philippe Gevaert, Belgium

11:02 66 *Correlation between basophil responsiveness and nasal symptoms following antigen challenge or seasonal exposure in grass allergic patients*
Peter A. Würtzen, Denmark

11:19 67 *Susceptibility of atopic dermatitis (AD) model mice to vaccinia and herpes simplex viruses*
Yuko Kawakami, United States

11:36 68 *Aggregated IgG activates human synovial mast cells from rheumatoid arthritis of osteoarthritis patients through Fc gamma RI*
Yoshimichi Okayama, Japan

11:53 69 *Transplacental transmission of RSV*
Giovanni Piedimonte, United States

12:10 70 *The effects of inhaled interferon β on viral exacerbations in asthma*
Stephen T. Holgate, United Kingdom

12:30 – 13:30 Paul Kallós Lecture
The Unsolved Mysteries of Antigen Presentation
Chairperson: John Bienenstock, Canada
Emil Unanue, MD
Paul & Ellen Lacy Professor
Washington University School of Medicine
United States

Dr. Unanue was born and raised in Havana, Cuba and went to Medical School at the University of Havana. He came to the United States in 1960 and did Pathology training at the University of Pittsburgh. Subsequently, he did post-doctoral research training in immunopathology, with Frank J. Dixon at Scripps Research Institute. With Dixon, he worked on the mechanisms by which antibodies produce immunological renal injury (glomerulonephritis). Following four years of post-doctoral work, he went to London to continue research work, this time in cellular immunology. The work in London was done, from 1966 to 1968 at the National Institute for Medical Research, at Mill Hill. The Division head at that time was John Humphrey and his work was done under Brigitte Askonas. He was in the faculty of Harvard Medical School from 1970 until 1985. His laboratory at Harvard was in the Department of Pathology in the Medical School proper. By 1985, he moved, with my laboratory, to Washington University School of Medicine where he headed their Department of Pathology and Immunology until 2006. With several colleagues they created an immunology research program centered on the cellular and molecular basis of lymphocytes and phagocytes and their interactions, areas they believe are central for the understanding of immune regulation. He now continues leading his own research laboratory maintaining his research efforts in cellular immunology.

13:30 – 15:00 Lunch ..... Shilla Jeju Hotel

Wednesday, 17 October 2012 (continued)

17:00 – 19:00 **Poster Session 4 ..... Lotus Room**
Pathophysiology of Allergic Disorders and Inflammation
Chairpersons: Mitchell H. Grayson, United States
Gunnar Nilsson, Sweden

71 *Evidence for T cell independence of secondary IgE responses in allergic patients*
Elopy Sibanda, Zimbabwe

72 *Micro-array based analysis of allergic and non-allergic individuals over ten years suggests absence of spontaneous de novo IgE sensitizations in adults*
Christian Lupinek, Austria

73 *Development of an allergic rhinitis model induced by Japanese Cedar pollen in BALB/c mice as an experimental model*
Katsuyo Ohashi-Doi, Japan

74 *Mast cell dependent and independent airway hyperresponsiveness*
Gunnar Nilsson, Sweden

75 *Prostaglandin D2 augments IgE-dependent histamine release from mast cells through E prostaglandin receptor, EP3*
Hiroyuki Tanaka, Japan

76 *Role of histamine H_4R in bleomycin-induced pulmonary fibrosis*
Emanuela Masini, Italy

78 *Alteration of intestinal microbiota increases mortality to respiratory viral infection in an IFN-gamma dependent mechanism*
Mitchell H. Grayson, United States

17:00 – 19:00 **Poster Session 5 ..... Lotus Room**
Clinical Aspects of Allergic Disorders
Chairpersons: Stephen R. Durham, United Kingdom
Marianne van Hage, Sweden

79 *Serum 25-hydroxyvitamin D level and atopy in Korean population*
Byoung Whui Choi, Korea

80 *In preschool children with acute wheeze, human rhinovirus group C infection is the most common cause and the only virus associated with atopy*
Peter Le Souëf, Australia

81 *Effect of the selective COX-2 inhibitor etoricoxib on allergen-induced airway obstruction and profile of lipid mediator metabolomics in bronchial provocation of subjects with asthma*
Barbro Dahlén, Sweden

82 *Dose response relationship between ascaris sensitisation with aeroallergen sensitisation and airway hyperresponsiveness but not allergic diseases in an urbanising teenage African population*
Michael Levin, South Africa

83 *Different phenotypes of exercise induced bronchoconstriction and associated factors: 8-year retrospective analysis of free running provocation test*
Kyung-Up Min, Korea

84 *Monitoring asthma with a smart-phone application*
Yoon-Seok Chang, Korea

85 *A novel technique for house dust mite inhalation in an Allergen Challenge Chamber – A validation study in patients with allergic rhinitis*
Norbert Krug, Germany

86 *Peripheral T cell signature after nasal allergen challenge in allergic rhinitis*
Stephen R. Durham, United Kingdom

87 *The efficacy and safety of a short course of budesonide inhalation suspension via transnasal nebulization in chronic rhinosinusitis with nasal polyps: A randomized, placebo-controlled study with immunologic evaluation*
Luo Zhang, China

Wednesday, 17 October 2012 (continued)

88 *Alleviation of sneezing and nasal rubbing in a murine model of allergic rhinitis by intranasal administration of Semaphorin 3A*
Zenro Ikezawa, Japan

89 *Aberrant expression of Semaphorin 3A and nerve growth factor in the epidermis is associated with itch in the skin lesions of not only atopic dermatitis but also prurigo nodularis and psoriasis*
Zenro Ikezawa, Japan

90 *Improvement of dryness and pruritus in the aceton-induced dry skin model by oral administration of collagen tripeptide*
Zenro Ikezawa, Japan

91 *Atopic Dermatitis in SubSaharan Africa: Clinical and sensitization patterns in Amaxhosa-speaking patients in Cape Town, SA*
Peter Schmid-Grendelmeier, Switzerland

92 *Allergic and immunodeficiency findings in atopic dermatitis and hyper-IgE syndrome patients*
Andreas Wollenberg, Germany

93 *Hymenoptera venom allergy: ICAM-I is overexpressed and decreases after venom immunotherapy*
Vincenzo Patella, Italy

17:00 – 19:00 Poster Session 6 ..... Lotus Room
Dendritic Cells, Monocytes, Mast Cells and Granulocytes
Chairpersons: Thomas Bieber, Germany
Hirohisa Saito, Japan

94 *Association between regulation of thymic stromal lymphopoietin receptor expression on peripheral blood dendritic cells and LPS-induced inflammasome activation in healthy children*
Yuzaburo Inoue, Japan

95 *Granulocyte/macrophage colony-stimulating factor-dependent CD11b+ lung dendritic cells are required for induction of T helper 2 immunity to inhaled dust mite allergen*
Qian Zhou, Singapore

96 *Plasmacytoid dendritic cells and innate interferons constrain Th2 cytokine responses to rhinovirus: A regulatory mechanism with relevance to asthma*
John W. Upham, Australia

97 *CD1a-/CD207-/CD11c+ human dendritic cells bind Bet v 1 allergen within the oral mucosal tissue*
Jean-Pierre Allam, Germany

98 *A new tool for studying intracellular interaction of the dermal mast cells*
Torsten Zuberbier, Germany

99 *Expression of a prostaglandin D2 receptor, CRTH2 (chemoattractant receptor-homologous molecule expressed on TH2 cells) on human mast cells and potential relevance in allergic diseases*
Tae Chul Moon, Canada

100 *Mast cells control allergic skin inflammation in a chronic type IV hypersensitivity model in mice*
Marcus Maurer, Germany

101 *Angiopoietins and their tie receptors in human basophils and mast cells*
Gianni Marone, Italy

102 *Eicosapentaenoic acid and decosahexaenoic acid suppress Th2 cytokine expression through distinct pathways in RBL-2H3 basophilic cells*
Mirim Jin, Korea

103 *Induction of Th2 cytokines by active Def f 1 in mouse bone marrow-derived basophils*
Myung-hee Yi, Korea

104 *The roles of phosphoinositide 3-kinase gamma for human eosinophil functions*
Masahide Takeda, Japan

105 *Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion competent eosinophil granules*
Shigeharu Ueki, Japan

106 *MicroRNA-155 is essential for Th2 mediated allergen-induced eosinophilic inflammation in the lung*
Madeleine Rådinger, Sweden

Schedule of Events

Thursday, 18 October 2012

7:00 – 8:00 *"Life in Science" Breakfast Discussion* ..... Rose Room
Stephen T. Holgate, United Kingdom

7:30 – 13:30 Registration Open ..... Shilla Jeju Hotel

7:30 – 15:00 Speaker Preview Room Open ..... Ora Room

8:00 – 10:16 Oral Abstract Session 6
Dendritic Cells, Monocytes, Mast Cells and Granulocytes
Chairperson: Gianni Marone, Germany
Jean S. Marshall, Canada

8:00 107 *Dendritic cell-dependent epigenetic modifications in Foxp3 and IL-10 on regulatory T cells during immunotherapy*
Kari C. Nadeau, United States

8:17 108 *Direct recruitment of pro-inflammatory slan-dendritic cells by immune complexes in a model of allergic vasculitis*
Knut Schaekel, Germany

8:34 110 *MicroRNA dysregulation in alveolar macrophages and its relevance to defective innate immunity and disease severity in asthma*
Peter H. Howarth, United Kingdom

8:51 111 *T cell derived microvesicles induce mast cell production of IL-24: A possible link to inflammatory skin diseases*
Yoseph A. Mekori, Israel

9:08 112 *Differential responses of human mast cells following Dengue virus Rheovirus and KSHV infections*
Jean S. Marshall, Canada

9:25 113 *The transcription factor TFE3 is a major regulator of mast cell mediated allergic response*
Ehud Razin, Israel

9:42 114 *Mast cells and vitamin D_3: A 'D'-lightful regulatory interaction*
Michele A. Grimbaldeston, Australia

10:00 – 10:45 Coffee ..... Halla Hall Lobby

10:45 – 13:20 Oral Abstract Session 7
Dendritic Cells, Mast Cells, Monocytes and Granulocytes
Chairpersons: Francesca Levi-Schaffer, Israel
Hans-Uwe Simon, Switzerland

10:45 115 *Mast cell activation is suppressed following co-culture with differentiated primary bronchial epithelial cells*
Emily J. Swindle, United Kingdom

11:02 116 *Insights into FcεRI signal initiation through defined valency ligands*
Bridget S. Wilson, United States

11:19 117 *Activation phenotypes are enhanced in interacting mast cells and eosinophils: Functional implications of the allergic effector unit*
Francesca Levi-Schaffer, Israel

11:36 118 *Alternative mechanisms of eosinophilopoiesis: Environmental factors influence neonatal immunity through alteration of cord blood progenitor CD34+ cell responses*
Pia-Lauren Reece, Canada

11:53 119 *Differing mechanisms for Siglec-F and Siglec-8-induced eosinophil apoptosis*
Bruce S. Bochner, United States

12:10 120 *Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps required for efficient killing of Staphylococcus epidermitis*
Dagmar Simon, Switzerland

12:27 121 *Regulated formation and functions of eosinophil lipid bodies in allergy and innate immune responses to infection*
Patricia Bozza, Argentina

12:44 122 *Functional aggregation of major basic protein in eosinophil granules and inflamed tissues*
Hans-Uwe Simon, Switzerland

13:01 123 *Critical role of P1-Runx1 in mouse basophil development*
Stephen J. Galli, United States

Thursday, 18 October 2012 (continued)

13:30 – 15:00 Lunch ..... Shilla Jeju Hotel

16:30 – 17:00 CIA Business Meeting ..... Halla Hall
All CIA members are asked to attend.

17:00 – 19:00 Poster Session 7 ..... Lotus Room
Urticaria and Angioedema
Chairperson: Marcus Maurer, Germany

124 *New-generation, fast-disintegrating taste-masked epinephrine sublingual tablets: Preclinical study*
F. Estelle R. Simons, Canada

125 *Confirmation of the greatest sensibility and specificity of basophils autoinduced degranulation test in the diagnosis of chronic autoimmune urticaria*
Massimo Caruso, Italy

126 *Clinical features of contrast media anaphylaxis*
Min-Suk Yang, Korea

17:00 – 19:00 Poster Session 8 ..... Lotus Room
Allergens and Diagnosis of Allergy
Chairpersons: Chein-Soo Hong, Korea

127 *Low proteolytic stability - One of the driving forces of allergenicity?*
Fatima Ferreira, Austria

129 *Dog saliva - An important source of dog allergens*
Marianne van Hage, Sweden

130 *Art v 3, the non-specific lipid transfer protein of mugwort pollen: Monoclonal antibodies to localize conformational human IgE epitopes on the crystal structure of an allergen*
Gabriele Gadermaier, Austria

131 *Cloning of cDNA and IgE reactivity of a-amylase from house dust mite, dermatophagoides farinae*
Chung-Ryul Kim, Korea

132 *Influence of temperature and buffer composition on the stability of house dust mite extract*
Kyoung Yong Jeong, Korea

133 *The IgE and IgG antibody binding profile of the known spectrum of protein cat allergens*
Wayne R. Thomas, Australia

134 *Three more case studies corroborate suspected lack of specific IgE in diclofenac hypersensitivity*
Martin Himly, Austria

17:00 – 19:00 Poster Session 9 ..... Lotus Room
Conventional and Novel Biomarkers of Asthma and COPD
Chairpersons: Jeffrey Drazen, United States
Stephen T. Holgate, United Kingdom

135 *Prevention of allopurinol induced severe cutaneous adverse reactions in patients with renal insufficiency by screening test for HLA-B*5801: Prospective study*
Hye-Ryun Kang, Korea

136 *Class 3 food allergy induced by contact sensitization of foods*
Zenro Ikezawa, Japan

137 *YKL-40 level in induced sputum is an acute phase biomarker in allergic airway inflammation*
Jung-Won Park, Korea

138 *Discovery of sputum biomarker for neutrophilic inflammation in severe uncontrolled asthma*
Choon-Sik Park, Korea

139 *Sputum interleukin 6: A biomarker for stratified therapy in treatment-resistant severe asthma*
Monica Salagean, United Kingdom

Thursday, 18 October 2012 (continued)

17:00 – 19:00 **Poster Session 10** ..... **Lotus Room**
Allergen Specific Immunotherapy
Chairpersons: Motohiro Ebisawa, Japan
Rudolf Valenta, Austria

140 *First in man immunotherapy study with the novel grass pollen allergy vaccine BM32 using the Vienna Challenge Chamber*
Rainer Henning, Austria

141 *Skin test evaluation of a new vaccine against grass pollen allergy based on recombinant fusion proteins consisting of hepatitis B PreS and peptides from four major grass pollen allergens*
Verena Niederberger, Austria

142 *Efficacy and safety of oral immunotherapy for food-induced anaphylaxis*
Motohiro Ebisawa, Japan

143 *Diamine oxidase: A novel biomarker for monitoring ex-vivo allergen-induced basophil reactivity following grass pollen immunotherapy*
Mohamed H. Shamji, United Kingdom

144 *Two year follow up after rush oral immunotherapy in hen's egg-induced anaphylactic children*
Katsuhito Iikura, Japan

145 *Enhancement of the frequency and function of IL-10-secreting type I T regulatory cells after one year of cluster allergen-specific immunotherapy*
Chengshuo Wang, China

17:00 – 19:00 **Poster Session 11** ..... **Lotus Room**
Treatment of Immune Disorders
Chairpersons: Mark Larche, Canada
Peter Weller, United States

147 *Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation*
Harissios Vliagoftis, Canada

148 *Metformin attenuates lung inflammation and remodeling via activation of adenosine monophosphate-activated protein kinase*
Hee-Bom Moon, Korea

149 *Human umbilical cord blood-derived mesenchymal stem cells suppress allergic airway inflammation in murine asthma model*
Cho Sang Heon, Korea

150 *Improved patients' hand grip strength with pMDI by using three fingers pinch method*
Jae Won Jeong, Korea

17:00 – 19:00 **Poster Session 12** ..... **Lotus Room**
Networks
Chairpersons: Sven Erik Dahlén, Sweden
Judah Denburg, Canada

151 *The Canadian Allergy, Genes and Environment Network: AllerGen NCE*
Judah A. Denburg, Canada

152 *GA²LEN - Global Allergy and Asthma European Network of Excellence*
Torsten Zuberbier, Germany

153 *Network presentation: CfA-The Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden*
Sven-Erik Dahlén, Sweden

154 *Christine Kuehne-Center for Allergy Research and Education (CK-CARE): Davos – Munich – Zurich*
Johannes Ring, Germany

155 *European Mast Cell and Basophil Research Network (EMBRN)*
Gunnar Nilsson, Sweden

156 *COST Action BM1007: Mast cell and basophils - Targets for innovative therapies*
Marcus Maurer, Germany

Thursday, 18 October 2012 (continued)

19:30 – 23:00 Gala Dinner ..... Shilla Jeju Hotel

Friday, 19 October 2012

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

7:30 – 13:00 Registration Open ..... Shilla Jeju Hotel

7:30 – 12:30 Speaker Preview Room Open ..... Ora Room

8:00 – 9:00 CIA Council Meeting ..... Lily Room

9:00 – 10:25 Oral Abstract Session 8 ..... Halla Hall
Dendritic Cells, Monocytes, Mast Cells and Granulocytes
Chairpersons: Hajime Karasuyama, Japan

9:00 157 *Critical roles for basophils in allergy and protective immunity as revealed by the basophil-engineered mice*
Hajime Karasuyama, Japan

9:17 158 *Gene expression profiles in human basophils after exposure to immobilized human IgG*
Kenji Matsumoto, Japan

9:00 – 10:25 Oral Abstract Session 8 ..... Halla Hall
Allergens and Diagnosis of Allergy
Chairpersons: Hajime Karasuyama, Japan

9:34 159 *IgE responses to galactose-a1, 3-galactose; involvement of tick Ixodes ricinus in red meat allergy*
Carl Hamsten, Sweden

9:51 160 *Increased hyperoxidized peroxiredoxins in peripheral blood mononuclear cells of asthma patients and polymorphisms in PRDX6 gene are associated with asthma severity*
Hyouk-Soo Kwon, Korea

10:08 161 *Serum periostin levels are correlated with decline of pulmonary function in asthma patients*
Kenji Izuhara, Japan

10:30 – 11:00 Coffee ..... Halla Hall Lobby

11:00 – 12:45 Oral Abstract Session 9 ..... Halla Hall
Treatment of Immune Disorders and Allergen-Specific Immunotherapy
Chairpersons: Robyn O'Hehir, Australia
Thomas A.E. Platts-Mills, United States

11:00 162 *Recombinant fusion proteins consisting of hepatitis PreS-fused non-allergenic Bet v 1 peptides focus blocking IgG towards IgE epitopes and shift T cell responses to a tolerogenic phenotype*
Katharina Marth, Austria

11:17 163 *Fel d 1 peptide immunotherapy for cat allergy is associated with IL-10 production to multiple cat allergens*
Mark Larche, Canada

11:34 164 *Design and characterization of a T cell epitope-based peptide immunotherapy for peanut Allergy*
Robyn E. O'Hehir, Australia

11:51 165 *Suppression of allergen-induced cutaneous late responses by repeated low dose intradermal allergen injection*
Stephen J. Till, United Kingdom

12:08 166 *Targeting IgE: A novel therapeutic option in severe atopic eczema*
Markus Ollert, Germany

12:25 167 *A neuro-endocrine anti-inflammatory pathway: Translation from rats to humans*
Dean Befus, Canada

Accreditation Statement

The 29th Symposium of the Collegium Internationale Allergologicum is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The 29th Symposium of the Collegium Internationale Allergologicum is designated for a maximum of 22 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

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Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

Learning Objectives

1. Updating basic, translational, and clinical science information about the origins, pathology, and clinical consequences of disorders in the fields of allergy, asthma, and clinical immunology.
2. Updating clinical information that will help to advance efforts to improve the prevention, clinical management, and, ultimately, cure of disorders in the fields of allergy, asthma, and clinical immunology.
3. Updating information about differences among the clinical approaches in different nations to improve the prevention, clinical management, and, ultimately, cure of disorders in the fields of allergy, asthma, and clinical immunology, so that it may be possible to agree on best practices for such efforts.

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ALK-Abelló, Salary

Oral Abstracts Session 1

1

Molecular genetics of atopic eczema

Johannes Ring and Stephan Weidinger

Department Dermatology and Allergy Biederstein, Christine Kühne-Center for Allergy Research and Education (CK-CARE), Technische Universitaet Muenchen, Munich and Department of Dermatology, Venerology and Allergology, University Kiel

Atopic diseases (asthma, rhinoconjunctivitis and eczema) have been found to be strongly genetically influenced, both from clinical experience and from molecular genetic studies. Atopic eczema (AE) is a complex trait, which results from the interaction of heritable and environmental factors. An important breakthrough in understanding the genetics of AE occurred with the identification of null mutations in the gene encoding filaggrin (FLG) leading to skin barrier dysfunction. Filaggrin deficiency due to inheritance of a single *FLG* mutation affects 10% of the general population, who suffer from dry and scaly skin and are at strongly increased risk to develop atopic eczema. The skin barrier defect associated with filaggrin mutations also increases the risk for food allergy, and appears to play an important role in the progression from eczema to allergic airway diseases. Yet FLG cannot explain the whole pathophysiology of AE. Studies on skin and immune function in "healthy" FLG-+ individuals are on the way.

In the past years, the advent of genome-wide association studies (GWAS) has revolutionized the discovery of genetic variants associated with complex diseases. The first GWAS on AE carried out on German, French, and East European cases resulted in the identification of a robust signal on chromosome 11q13.5. The gene or gene product affected by the respective risk allele remains to be identified and functionally characterized, but it appears to be of relevance for epithelial inflammation in diverse chronic inflammatory diseases.

More recently, a meta-analysis of GWAS was carried out in more than 10,000 AE cases and 40,000 healthy individuals within the framework of the "EAGLE" Consortium (EArly Genetics and Lifecourse Epidemiology). Three polymorphisms showed consistent associations: rs479844 upstream of *OVOL1* and rs2164983 near *ACTL9*, genes which have been implicated in epidermal proliferation and differentiation, as well as rs2897442 in *KIF3A* within the cytokine cluster on 5q31.1. Finemapping and conditional analyses indicated that there are two distinct signals at this locus, one within the *RAE50/IL13* region and one within the *IL4/KIF3A* region, while expression quantitative trait analysis suggests both of these signals are associated with transcript levels of IL13. Finally, a GWAS in a Chinese Han population identified two novel loci, one of which also showed evidence for association in a German sample (rs6010620, 20q13.33).

These important results from molecular genetics point towards key disease pathways affecting both epithelial barrier function and immune responses. However, much remains to be learned about how the genome influences AE risk and pathobiology, and much remains to be done before genetic findings can finally be translated into an improved classification and the development of new and more targeted diagnostic or therapeutic strategies.

2

Asthma phenotypes: Heterogeneity and genomics

Eugene Bleecker, Xingnan Li, Wendy E. Moore, Greg Hawkins, Rebecca E. Slager, Deborah A. Meyers

Background: Heterogeneity in asthma is poorly characterized by guideline severity classifications. Severe Asthma Research Program (NHLBI: SARP) subjects have been classified into 5 clinical clusters using unsupervised cluster analysis and 34 variables (Moore 2010). Eleven variables allow assignment of these clinical clusters in other cohorts. We hypothesize that these clinical clusters could be further refined using biomarkers such as sputum inflammatory cell counts and genetic variants.

Methods: Factor analysis was performed to select 15 variables (baseline and maximal FEV1, age, race, gender, BMI, age of onset, asthma duration, medication use and health care utilization, % eosinophils and %neutrophils from blood and sputum), followed by an unsupervised cluster analysis on 423 asthmatics. To identify genes associated with the clusters, genome wide association analysis was performed on 785 asthmatics.

Results: Incorporating the blood and sputum eosinophil data resulted in four clusters with similar characteristics to four of the five clinical clusters. All four previously reported inflammatory cell profiles (high or low eosinophils and/or neutrophils)(Hastie 2010) were present in all clusters consisted with significant heterogeneity in the inflammatory mechanisms that may underlie these groupings. Genes associated with lung development (*HGF*), hypoxia response (*CITED2*), and airway inflammation (*IL1RAP*) were associated with cluster based asthma severity (P= ~10-5). Lung function genes: *HHIP* and *IL6R* were consistently associated with asthma severity clusters in non-Hispanic whites and African-Americans. Individual IL4/13 pathway variants were associated with the clinical clusters in non-Hispanic whites and African-Americans, and atopic asthma clusters of increasing severity. In a cumulative analysis of significant SNPs, increasing number of risk genotypes predict increasing likelihood of severe atopic asthma.

Conclusions: Use of unsupervised cluster approaches improves characterization of asthma severity and heterogeneity. Sputum clusters have overlapping characteristics with the original clinical clusters. Genetic variation is associated with clinical asthma severity clusters supporting the importance of understanding the biology of sub-phenotypes in asthma. (Supported by NHLBI: RC2 HL101487, U10 HL109164).

3

Asthma-associated variants in IL13 upregulate human IL4 expression in mice humanized for the Th2 cytokine locus

Donata Vercelli, Vadim Pivniouk, Oksana Pivniouk

Background: A block of 9 single nucleotide polymorphisms (SNPs) in human (h) IL13 is strongly associated with increased susceptibility to allergy and asthma in several human populations of distinct ethnicity.

Methods: To study the functional significance of these SNPs in vivo, we generated mice that carry a 160 kb BAC5 transgene (TG) encompassing a non-polymorphic hTh2 cytokine locus (HapI). A HapII TG was engineered by introducing the asthma-associated IL13 SNPs into BAC5. hTh2 cytokine expression was faithfully regulated in HapI and HapII mice.

Results: To evaluate the effects of asthma-associated SNPs, we compared hTh2 cytokine expression by polarized Th2 cells from Hap I and Hap II mice. hIL13 secretion was marginally upregulated in Hap II mice but, surprisingly, hIL4 protein and mRNA levels were dramatically increased. Increased hIL4 expression in carriers of IL13 SNPs was confirmed in humans. Mouse Th2 cytokine expression was unaffected.

To elucidate the mechanisms underlying the effects of hIL13 SNPs on hIL4 expression, we performed a high-resolution analysis of DNA methylation at the Th2 cytokine locus of HapI and HapII Th2 cells. Several differentially methylated regions were identified. Notably, the HSIV element, which is located ~25 kb 3' of the IL13 SNPs and acts as an IL4 silencer in mice, was significantly hypermethylated at three consecutive CpG sites in HapII Th2 cells.

Conclusions: Our results point to novel long-range effects of the IL13 SNPs on HSIV-dependent IL4 silencing, which may increase susceptibility to Th2-dependent allergic disease.
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4

Genome-wide association studies of total serum IgE levels in asthma

Xingnan Li, Eugene Ronald Bleecker, Deborah A. Meyers

Background: Genome-wide association studies (GWAS) of asthma and total serum IgE levels, including our studies [1,2], have identified three shared genes for asthma and IgE: *RAD50-IL13*, *HLA-DR/DQ* and *C11orf30-LRRC32* regions.

Methods: In this study, GWAS of total serum IgE levels in asthmatic subjects was performed in non-Hispanic white populations from SARP, CSGA, and TENOR studies (n=1,042), and in African American populations from SARP and CSGA studies (n=324) using PLINK software. Meta-analyses of P values were performed by using METAL software.

Results: Our study confirmed several candidate genes: *HLA-DQA1* (rs9271300: $P = 6x10^{-5}$), *HLA-G* (rs2975033: P = 0.007), *FCER1A* (rs2251746: P = 0.01), *STAT6-LRP1* (rs715948: P = 0.005), *RAD50-IL13-IL4* (rs2243204: P = 0.002), and *IL4R* (rs3024536: P = 0.04). Our study also indicated several novel candidate genes for total serum IgE levels in asthmatics: Adherens junctions associated protein 1 (rs780606 in 5' of *AJAP1*: $P = 7x10^{-5}$ and 0.04 for Whites and African Americans, respectively) and RNA-specific adenosine deaminase B2 (rs1046914 at 3'UTR of *ADARB2*: P = 0.008 and $1x10^{-5}$ for Whites and African Americans, respectively).

Conclusions: Candidate genes associated with total serum IgE levels in general populations are also associated with IgE in asthmatics. Further replication of novel candidate genes in asthmatics is on process.

(This abstract is funded by HL87665 and HL101487)
[1] Li X, et al. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol* 2010; 125:328-335. e11.

[2] Li X, et al. The C11orf30-LRRC32 region is associated with total serum IgE levels in asthmatic patients. *J Allergy Clin Immunol* 2012; 129:575-578. e1-9.

5

Role of genetics in severe asthma

Deborah A. Meyers, Rebecca E. Slager, Xingnan Li, Eugene Ronald Bleecker

Background: Genome-wide association studies (GWAS) in asthma, allergy and other common diseases have identified genes important in disease susceptibility, usually common variants where the overall contribution of each individual variant is relatively small. By utilizing the very well phenotyped mild to severe asthmatics in the NHLBI Severe Asthma Research Program (SARP), we have observed that the genes for asthma susceptibility differ from those for asthma severity and related phenotypes such as lung function (Li 2010). For example, the common SNP rs3751144 in the P2RX7 (purinergic receptor P2X ligand-gated ion channel 7) gene on chromosome 12 was associated with severity (P=2.21E-04) in a GWAS of SARP African-Americans (n=155 severe, 214 non-severe).

Methods: With new sequencing technology, it is now possible to examine the role of rare functional variants which may have a larger phenotypic effect. Exome sequencing analysis was performed in a subset of 191 SARP African-Americans to compare subjects with mild asthma to those with severe asthma (as part of the NHLBI Exome Sequencing Project). In addition, exome sequencing was performed for 20 SARP non-Hispanic white subjects with asthma to test the hypothesis that rare genetic variants in TH1 and TH2 pathway genes contribute to asthma severity.

Results: In the African-Americans, the lowest P values from single variant analysis were observed for three common variants including one missense variation, rs2230911 (T357S; P=2.50E-05) in P2RX7. Even in the small sample of white asthmatics, novel amino acid changes were identified: IL12RB2 (R475W), JAK3 (Y1023H) and IL1RL1 (R396H). Subjects with increased numbers of TH1 rare variants were more likely to report lower quality of life scores, including increased activities limited by asthma (P=0.047). Subjects with any TH2 rare variants were significantly more likely to experience severe year-round allergy symptoms (P=0.02) and a trends towards increased total serum IgE levels (Geometric mean 77.9 vs. 26.9) and increased mean number of positive skin test reactions (3.40 vs. 2.25) was observed, compared to subjects with no rare TH2 variants.

Conclusions: These findings will be extended using the Illumina Exome chip but provide preliminary results demonstrating the potential importance of rare functional genetic variants in asthma severity.

6

Genome-wide association study of aspirin-intolerant asthma in the Japanese population

Mayumi Tamari, Tomomitsu Hirota, Michiaki Kubo, Yusuke Nakamura

Background: Bronchial asthma is a heterogeneous disease with many etiologic factors and clinical characteristics. Aspirin-intolerant asthma (AIA) is a common clinical presentation of aspirin hypersensitivity, and this acute reaction is elicited via cyclooxygenase inhibition by non-steroid anti-inflammatory drugs. Although association studies for candidate genes have been conducted, the influences of genetic factors in AIA have not been fully elucidated. Genome-wide association studies (GWAS), which comprehensively assess genes related to multifactorial diseases, have enhanced our understanding of human allergic diseases. We conducted a GWAS for AIA in a Japanese population to discover the genes and cellular pathways that might have an effect on susceptibility to AIA.

Methods: We recruited 153 patients with AIA and 3302 controls who did not have bronchial asthma, atopic dermatitis or allergic rhinitis. All patients with AIA were diagnosed by physicians and were documented to have histories of asthmatic attacks following the ingestion of more than two different kinds of non-steroidal anti-inflammatory drugs or to have had a positive reaction to systemic aspirin challenge. We conducted the GWAS for AIA in the Japanese population using Illumina HumanHap 550v3 and 610 Quad BeadChips. We excluded SNPs with minor allele frequencies of less than 0.01 in both cases and controls. We conducted exact Hardy-Weinberg equilibrium analysis, and SNPs with $P < 10^{-6}$ in controls were excluded from the analysis.

Results: After quality-control filtering, we generated a quantile-quantile plot using the results of a Cochran-Armitage test with a total of 433,543 SNPs having call rates ≥99% in both cases and controls. The genomic inflation factor (λ_{GC}) was 1.016. We observed a strong association at rs7277220 on chromosome 21q21.2 ($P=8.72 \times 10^{-8}$) and a total of 98 SNPs showed $P < 1 \times 10^{-4}$.

Conclusion: Although we could not detect any associations at genome-wide significant levels, we identified a total of 98 SNPs for which $P < 1 \times 10^{-4}$ in our GWAS. Further studies of these loci are necessary for better understanding of the genetic etiology and pathophysiology of AIA.

7

Molecular genetic mechanisms of eosinophil activation in patients with ASA exacerbated respiratory disease

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Background: The ASA exacerbated respiratory disease (AERD) is the major phenotype of ASA hypersensitivity with 4.3%–12% prevalence of adult asthma. The clinical severity of AERD patients is moderate to severe asthma with high prevalence of chronic rhino-sinusitis complicated by polyp. Intense eosinophil infiltration was noted in upper and lower airways. The recent genetic polymorphism studies of AERD have focused on two lines of enquiry: 1) leukotriene- and prostanoid-related genes including the lipoxygenase (LO) pathway and cysteinyl leukotriene receptors, and 2) eosinophil-related genes.

Methods: In this study, we investigated the molecular genetic mechanisms involved in eosinophil activation in AERD patients, compared with two control groups, ASA tolerant asthma and healthy controls. The phenotype of AERD was confirmed by lysine ASA bronchial challenge test. Genotype of candidate genes was analyzed using a primer extension method.

Results: The T allele of CRTH2 -466T>C polymorphism was higher in AERD patients than in control groups and could increase serum and cellular eotaxin-2 production by lowering the CRTH2 expression, leading to eosinophilic infiltration. The mutation rate of CCR3 at -520 T>C was higher in AERD patients than in control groups in whom mRNA expression increased after ASA challenge, leading to eosinophil activation. The promoter polymorphisms of ALOX 15(-427G>A, -272C>A, -217G>C) were associated with the phenotype of AERD and could increase 15-LO activity and increased peripheral eosinophil count. The promoter polymorphism at -5993 G>A of IL-5 receptor gene (IL5R) was associated with severe asthma group of AERD patients with higher peripheral eosinophil count, as the A allele could increase serum eotaxin level and IL5R expression on eosinophils, leading to eosinophil activation in AERD patients.

Conclusion: We suggest that the genetic polymorphisms of CRTH2, CCR3, ALOX15 and IL5R could involve in eosinophil activation in upper and lower airway inflammation of AERD patients. These findings may provide potential biomarkers for predicting drug responses such as anit-IL5 and IL5R antibodies or CCR3 and CRTH2 antagonists, and potential targets for new drug development.

8

Climate change, pollen and allergy

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Background: Global warming is a physico-meteorological fact that – among other aspects – will also affect human health. Apart from cardiovascular and infectious diseases, allergies seem to be at the forefront of the sequelae of climate change. Climate change will lead to elevated CO_2 and ozone levels with possible effects upon neophyte growth and pollen allergenicity. It is the aim of this project to investigate the impact of various natural and anthropogenic factors under different climate change scenarios upon the allergenicity of pollinating plants.

Methods: In order to investigate the impact of urbanization catkins from birch trees were sampled from urban and rural sites in Germany, pollen were isolated and NO_2 and O_3 exposure was measured at sites of sampling. In aqueous pollen extracts major allergen Bet v 1 as well as pollen-associated liquid mediators (PALMs) were measured. The immunomodulatory potential was estimated by inhibition of dendritic cell IL-12 response, the proinflammatory effects by neutrophil chemotaxis assays, resp.

Ragweed plants were grown under controlled conditions simulating various climate change scenarios in greenhouse chambers and exposed to control air, different concentrations of ozone and various degrees of temperature and humidity. 454-sequencing of two different pollen cDNA libraries for pollen transcriptome analyses, LC-MS/MS of aqueous pollen extracts were performed. *In vivo* effects of differently exposed pollen were analyzed in mice sensitization models and in human volunteers using skin prick tests and nasal provocation tests.

Results: Climate change parameters, especially elevated ozone, influenced the allergenic potential of ambrosia pollen as shown by transcriptome analysis and protein measurement in extracts. Large scale 454-sequencing allowed the identification of stress-related transcripts in mature pollen, among them Amb a 1 and other allergens. In mice climate change factors such as CO_2 or drought, but not ozone, increased the IgE-producing capacity of ragweed pollen. In humans skin prick test and nasal provocation tests were enhanced when extracts from altered pollen were applied.

Pollen grains from birch trees exposed to elevated ozone levels contained higher levels of Bet v 1 than pollen from lower ozone-expose birch trees. Pollen from higher ozone-exposed birch trees induced higher neutrophil chemotaxis but were less potent in inhibiting the dendritic cell interleukin-12 response. Pollen from high ozone-exposed trees induced enhanced wheal-and-flare reactions in skin prick tests than pollen from low ozone-exposed trees.

Conclusion: Climate change parameters such as increase in temperature and concomitant CO_2 concentration together with ozone and other air pollutants have a direct effect on allergenicity of pollinating plants as measured in ambient air, *in vitro* under greenhouse conditions as well as *in vivo* in animal experiments and humans. Through the effects of climate change, in the future plant growth may be influenced in a way that more new and altered pollen are produced, thus contributing to a further increase in allergy prevalence and symptom severity.

9

Metagenomics of microbiota and extracellular vesicles in indoor dust and stools

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Background: The role of infectious agents in the etiology of inflammatory diseases once believed to be non-infectious is increasingly being recognized. Biological contaminants in indoor air can induce chronic inflammatory pulmonary disorders, such as asthma and COPD and also gut microbiota are related with pathogenesis of inflammatory bowel disease (IBD). However, owing to the complexity of microbiota in our environments, our understanding of the roles of commensal and pathogenic bacteria in establishing a healthy intestinal barrier and in mucosal inflammatory disease pathogenesis is evolving only slowly. Metagenomics, sequencing of DNA from an environmental sample, offers a powerful means to better understand the complex relationships between hosts and their bacterial inhabitants. Recently, we found that bacteria-derived extracellular vesicles (EV), which contain 16S DNA and 16S RNA, are causally related with inflammatory diseases.

Method: We evaluated compositions of microbiota and EV in indoor dust and stools using 16S DNA sequencing.

Results: Microbiota compositions in indoor dust revealed the presence of gram-negative and gram-positive bacteria, with most sequences (> 90%) related to just five genus: Pseudomonas (61.6%), Enterobacter (13.6%), Acinetobacter (7.0%), Leclercia (4.5%), and Staphylococcus (0.9%). In terms of EV compositions in indoor dust, five genus were predominant: Pseudomonas (83.6%), Enterobacter (6.0%), Nicotiana (2.0%), Acinetobacter (1.9%), and Staphylococcus (1.3%). Meanwhile, the present study showed that gut microbiota compositions were similar between stools from healthy and chemical-induced IBD mice. However, the compositions of bacteria-derived EV in stools were distinct in the two groups.

Conclusion: These data indicate that microbiota-derived EV are present in our environments and may be related to pathogenesis of inflammatory diseases.

Oral Abstracts Session 2

10

Amphiregulin, a potential survival factor, is produced by $CD4^+$ T cells following NMDA receptor activation

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Background: Increase in epidermal growth factor receptor (EGFR) expression in the airways is associated with severe and corticosteroid-resistant asthma. Recently, several reports suggested that amphiregulin, a member of the epithelial growth factor (EGF) family, may be closely associated with airway remodeling in corticosteroid resistance. Amphiregulin is also a product of mast cells, eosinophils, basophils, and bronchial epithelial cells. Glutamate is the most common neurotransmitter in the central nervous system that signals partially through *N*-methyl-*D*-aspartate (NMDA) receptors. The impact of NMDA receptors in neuroscience is convincing in various neurodegenerative conditions including Alzheimer's disease, HIV dementia, epilepsy and Huntington's disease. We have shown that activated $CD4^+$ T cells express functional NMDA receptors.

In this study, we hypothesize that signaling via NMDA receptors enhances amphiregulin expression and secretion from $CD4^+$ T cells, and contributes to $CD4^+$ T cell survival.

Methods: $CD4^+$ T cells were isolated from PBMC from consenting adult healthy donors, and activated with anti-CD2/3/28 antibodies. Expression levels of mRNA and protein were determined using quantitative real-time PCR and ELISA, respectively. Cell viability (metabolism) was detected by MTT assay. Promoter analysis was performed ***in silico***, using the online software, Transfac, Matrix Search for Transcription Factor Binding Sites provided by Biobase.

Results: Glutamate treatment *in vitro* significantly enhanced amphiregulin expression (mRNA) and production (protein) from activated human $CD4^+$ T cells. Also, exogenous amphiregulin treatment promoted $CD4^+$ cells survival. The effect of amphiregulin to support cell survival was clearer when the cells were cultured in the presence of IL-4. *In silico* promoter analysis indicated that amphiregulin gene sequence has the highest theoretical number of GATA-3 binding sites in its promoter sequences than other EGFR agonists. Real-time PCR data also showed that amphiregulin expression level correlated positively with IL-4 expression levels in activated $CD4^+$ T cells.

Conclusion: We detected amphiregulin production from $CD4^+$ T cells, which is enhanced following NMDA receptor activation. Evidence involving GATA-3 and IL-4 indicated that amphiregulin may be differentially expressed in association with Th2, but not Th1 cells. Thus, we suggest that amphiregulin may be a potential survival factor for Th2 cells, regardless of cytokine signaling.

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Induced regulatory T cells (Treg) are distinctly superior to natural Treg of the same allergen specificity in their abilities to induce asthma tolerance

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Background: Naturally-occurring regulatory T cells (nTreg) are critical to the maintenance of tolerance to self-antigens, and arise for this purpose within the thymus as $CD4^+CD25^+Foxp3^+$ T cells. Another type of $CD4^+CD25^+Foxp3^+$ Treg can also be induced (iTreg) in the periphery from naïve T cells under the influence of TGFβ and retinoic acid, or from committed T effector cells (Teff) under the influence of IL-10-differentiated dendritic cells (DC10). The recognition that Treg are potently immunosuppressive gave rise to proposals that these cells could be used therapeutically - indeed, passive transfer of Treg can suppress pathology in autoimmune and allergic diseases, for example. However an important issue in Treg immunotherapy is which sub-population of Treg to employ in any specific context. There has been much speculation that antigen-specific induced Treg would be superior to non-specifically expanded nTreg in clinical settings because these cells would be expected to target antigen-specific (i.e., pathogenic) T cells with much greater efficiency. As intuitive as this may seem, there is a lack of compelling evidence to support this proposal.

Methods: Herein we assessed the relative efficiency with which $CD25^+Foxp3^+$ nTreg or iTreg of identical TCR specificity (i.e., from OVA-TCR transgenic OT2 mice) could induce tolerance in a mouse model of asthma. The iTreg were so induced either by culture of Teff cells from asthmatic OT2 mice with OVA-presenting DC10 or by injecting these Teff cells into DC10-treated OVA-asthmatic OT2 mice. We purified nTreg from allergen-naïve OT2 mice and also cultured them with DC10 before use, or co-injected them into DC10-treated recipients. Both populations were then sorted and assessed for their therapeutic efficacy.

Results: DC10 engaged nTreg in a cognate fashion in FRET assays, and these nTreg subsequently reduced Th2 effector T cell responses to OVA by 40-50% *in vitro*, while DC10-induced iTreg diminished these responses by 73-83%. Neutralization of IL-10, but not TGFβ, eliminated the suppressive activities of iTreg, but not the nTreg. Delivery of $5x10^5$ purified nTreg reduced airway eosinophil and IL-4/IL-5 responses of asthmatic recipient mice by 4-26%, but did not affect airway hyperresponsiveness (AHR) or IgE levels, while equal numbers of iTreg reduced all airway responses to allergen challenge by 80-96% and fully normalized AHR.

Conclusions: These data suggest that iTreg and nTreg of the same antigen specificity employ distinct mechanisms to effect tolerance and indicate that iTreg are substantially more tolerogenic in asthmatic recipients

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Induction of Foxp3+ regulatory T cells by Lactobacillus rhamnosus: A critical role for heme oxygenase 1

Paul Forsythe, Khalil Karimi, Nalaayini Kandiah, Jessie Chau, John Bienenstock

Introduction: Increasing awareness of the role of intestinal commensal bacteria in the development and modulation of the immune system has lead to great interest in the therapeutic potential of bacteria based strategies for a range of immune disorders. The anti-inflammatory effects of certain commensal bacteria have been linked to their ability to induce regulatory T cells (Treg). However many aspects of the mechanisms underlying Treg induction by these bacteria remain obscure. We investigated the ability of a Lactobacillus species to induce Foxp3+ Treg in GALT and mesenteric lymph nodes. We further investigated the potential role of dendritic cells and the immunomodulatory enzyme hemeoxygenase-1 (HO-1) in mediating these responses.

Methods: Mice were administered $1x10^9$ *Lactobacillus rhamnousus* (JB-1) or *Lactobacillus salivarius* in MRS broth, or MRS broth only, via gavage, daily for 3, 5 or 9 days. Single cell suspensions of Peyer's Patches (PP) and mesenteric lymph node (MLN) were labeled for extracellular and intracellular markers and analyzed by FACS. Isolated cells were stimulated with CD3/CD28 and cytokine release determined. Mouse bone marrow derived dendritic cells were incubated with PKH26 labeled JB-1 and changes in HO-1 expression assessed by FACS. JB-1 exposed

dendritic cells were injected into the footpad of mice and Foxp3 expression determined in isolated popliteal lymph node cells.

Results: Oral treatment with *L. rhamnousus* (JB-1), but not *L.salivarius*, lead to a significant increase in the percentage of CD4+ T cells expressing Foxp3 in both the PP and MLN. This increase was greatest in the MLN and maximal after 5 days treatment (1.7±0.8% vs 15.3 ±3.5 % in control and LB treated respectively n=5, p<0.01) and was associated with a marked decrease in TNF and IFNγ release following stimulation of isolated MLN cells. JB-1 also lead to a significant increase in CD11c+ cells expressing HO-1 in the MLN (8.0±1.5 % vs 17.4±2.1 n=5, P<0.05). These changes could be mimicked by direct co-culture of bone marrow derived DC with LB. Experiments with fluorescence labeled JB-1 determined that fed bacteria were taken up by DC in the Peyer's patches and that HO-1 expression occurred almost exclusively in DC directly associated with the bacteria. DC exposed to JB-1 demonstrated regulatory function and adoptive transfer of these cells lead to an increased CD4+ Foxp3+ population in lymph nodes of recipient mice. Treatment of mice with a heme oxygenase inhibitor, chromium mesoporphyrin, abolished the increase in Foxp3+ CD4+ T cells induced by JB-1, but did not prevent the inhibition of IFNγ and TNF production by stimulated MLN cells.

Conclusion: Oral treatment with JB-1 induces HO-1+ regulatory DC in the MLN. Furthermore activity of HO-1 is critical to the induction of Foxp3+ Treg by this bacteria. However, additional, as yet unknown, pathways are involved in JB-1 mediated down-regulation of inflammatory cytokine production in the MLN. Given the extant literature suggesting a protective effect of increased HO-1 expression in models of asthma and inflammatory bowel disease it will be interesting to determine whether activation of this enzyme is an important element of beneficial effects described for certain commensal organisms in these systems.

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IL-35+CD4+CD25+Foxp3- regulatory T cells suppress Th2 responses and are induced following grass pollen-specific sublingual immunotherapy

Mohamed H. Shamji, Janice Layhadi, Daniela Achkova, Guy W. Scadding, Stephen R. Durham

Background: Grass pollen-specific immunotherapy involves the immunomodulation of allergen-specific T helper 2 cell (TH2) responses and induction of IL-10+ and/or TGF-β+CD4+CD25+ regulatory T cells (T regs). IL-35+CD4+CD25+Foxp3- T cells have recently been reported as a novel subset of regulatory T cells with modulatory characteristics. These regulatory T cells produce IL-35, which is a dimeric protein with two subunits, IL-12A and Epstein-Barr virus induced 3 (EBI3). Murine studies have revealed the role of IL-35 in suppressing allergic inflammation. We hypothesized that IL-35 suppresses grass pollen-specific Th2 responses following ex-vivo allergen stimulation. We further hypothesized that IL-35+CD4+CD25+Foxp3- Tregs may be induced following grass pollen-specific sublingual immunotherapy

Method: T effector cells (CD4+CD25-) obtained from grass pollen allergics (n=6) were purified from peripheral blood mononuclear cells by magnetic separation using CD4+CD25+CD127dim/- regulatory T Cell Isolation Kit. CD4+CD25- T cells were co-cultured with irradiated APC using 5ug/mL of *Phleum pratense* in the presence/absence 10ng/mL of recombinant human IL-35:Fc. T cell proliferative responses was measured by thymidine incorporation. Cytokine gene expression and protein levels levels were assessed by RT-PCR and Luminex MagPix assay, respectively.

Results: IL-35 significantly suppressed *Phleum pratense*-driven CD4+CD25- T cell proliferative responses (n=6; p=0.03). This suppression was associated with reduced IL-4 (p= 0.002), IL-5 (p=0.03). IL-9 (p=0.03) IL-13 (p=0.03) and an increase in IFN-g (p=0.02) and IL-10 (p=0.03) was also demonstrated. Furthermore, naïve CD4+CD45RA T cells stimulated with anti-CD3/CD28 in the presence of 10ng/mL of IL-35 for 9 days were polarised into IL-35+CD4+CD25+FOXP3- (p=0.01) and IL-10+CD4+CD25+ (p=0.03) and CD4+CD25+FoxP3+ CD127lo Tregs (P=0.001). These cells suppressed allergen-driven memory CD4+CD45RO+ proliferative responses (p=0.006). Interestingly, increased up regulation of IL-12p35 (p=0.01), EBI3 (p=0.04), Foxp3 (p=0.002) mRNA expression in allergen stimulated PBMCs in SLIT treated patients was demonstrated. A parallel increase in the proportion of IL-35+ (IL-12p35) and EBI3) CD4+CD25+FoxP3- (p=0.03) and CD4+CD25+FoxP3+CD127lo Tregs as measured by flow cytometry was also shown.

Conclusions: Our findings suggest that IL-35 suppresses grass pollen-driven T cell responses and is associated with the induction of IL-35+ and CD4+CD25^{hi}Foxp3+ Tregs. Moreover, IL-35+ T regs are induced following SLIT.

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In vitro differentiation and transcriptome profiling of human Th9 cells

David J. Cousins, Celine Parmentier, Tak Hong Lee

Background: Th9 cells are a recently described subset of T-effector cells that express interleukin-9 (IL-9) and IL-10. Experimental data derived from mouse models suggests that Th9 cells can develop in the presence of the Th2 cytokine IL-4 and transforming growth factor β(ΤΓΦβ)In mouse models IL-9 and Th9 cells have recently been implicated in the pathogenesis of allergic airways disease.

Objective: To determine whether human naïve T-cells could differentiate into a Th9 population and to characterise the Th9 cell gene expression profile.

Methods: Naïve human CD4+ T-cells were isolated from peripheral blood and differentiated *in vitro* under various conditions. Multicolour flow cytometry was used to phenotype IL-9 producing cells. Real-time PCR was used to examine expression levels of individual genes. Furthermore, to gain insight into factors that may be selectively expressed in human Th9 cells we have performed comparative microarray based transcriptome profiling.

Results: We show that human naïve CD4+ T-cells cultured with IL-4 and TGFβ develop into Th9 cells capable of expressing IL-9 in the absence of Th1 (IFNγ), Th2 (IL-4, IL-5, IL-13) or Th17 (IL-17) cytokines. In contrast to mouse Th9 cells the human IL-9 positive cells do not co-express IL-10. Several other features of human Th9 cells also appear to differ from their murine counterparts. The transcription factors PU.1 and IRF4 have both been implicated in mouse Th9 cell differentiation, we observed no expression of PU.1 by human Th9 cells. IRF4 was widely expressed in all T-helper cell lineages with no evidence for selective expression in the Th9 subset. In common with mouse Th9 cells we observe high-level expression of the IL-25 receptor IL-17RB. Transcriptome profiling identified 156 genes that were differentially expressed between Th2 and Th9 cells (>1.5 fold, p-value <0.05 including false discovery rate).

Conclusions: Our data show that human Th9 cells are induced in a similar fashion to those identified in murine systems but that several key features differ between species. Hierarchical clustering analysis suggests that Th9 cells have a more similar gene expression program to Th2 cells than to other human T-helper subsets.

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Human B regulatory 1 cells suppress antigen-specific immune responses and develop IgG4-producing plasma cells

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Background: IL-10-producing regulatory B cells suppress immune responses and the lack of regulatory B cells leads to exacerbated symptoms in mouse models of autoimmunity, transplantation and chronic infections. IgG4 is a characteristic antibody isotype induced in many human high dose antigen tolerance models. Due to its unique structural features IgG4 has anti-inflammatory potential.

Objective: In the present study, we characterized human inducible IL-10-secreting B regulatory 1 (Br1) cells and investigated their link to the isotype of the immunoglobulin that they produce after developing into plasma cells.

Methods: Human IL-10-secreting peripheral B cells were labeled using a cytokine secretion assay and purified by fluorescence activated cell sorting. IL-10-secreting and non-IL-10 secreting B cells were phenotypically and functionally characterized by whole genome expression analysis, flow cytometry, suppression assay and antibody production analysis. Furthermore B cells specific for the major bee venom allergen phospholipase A2 were isolated from beekeepers who developed immunological tolerance to bee venom antigens.

Results: Human Br1 cells expressed high CD25, CD71, and CD274, and low CD73 on their surface, and potently suppressed antigen-specific CD4+ T cell proliferation compared to IL-10- B cells. The class of the immunoglobulin produced by human Br1 cells isolated from healthy individuals is selectively confined to IgG4. Supporting these findings, B cells isolated from beekeepers that are specific for phospholipase A2 have increased expression of IL-10 and IgG4.

Conclusion: Our data show that two essential arrays of allergen tolerance; the suppressive B cell and IgG4 expressing B cell, are confined to Br1 cells in humans.

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Role of natural helper cells in the lung inflammation

Shigeo Koyasu

Natural helper (NH) cells produced IL-5, IL-13 and eotaxin in response to IL-33 or the combination of IL-2 and IL-25 (IL-2+IL-25). Administration of IL-33 or IL-2+IL-25 induced recruitment of NH cells and eosinophilia in the lung of Rag-$2^{-/-}$ mice while IL-25 alone did not induce these reactions. *Nippostrongylus brasiliensis* infection also resulted in the recruitment of NH cells and eosinophilia in the lung of Rag-$2^{-/-}$ mice but not in $\gamma_c^{-/-}$Rag-$2^{-/-}$ mice lacking NH cells. Adoptive transfer of NH cells restored the lung eosinophilia in $\gamma_c^{-/-}$Rag-$2^{-/-}$ mice upon helminth infection or IL-33 or IL-2+IL-25 administration. Naïve NH cells expressed CCR2 but IL-33 or IL-2+IL-25 stimulation down-regulated CCR2 and induced CCR4, enabling NH cells to migrate to the lung. After we reported NH cells, other groups also reported novel Th2 cytokine producer cells, such as MPP^{type2} cells and nuocytes induced in the lymph nodes. There are similarities and differences between these newly identified cell populations and NH cells. MPP^{type2} cells can differentiate to other myeloid cells, making this cell type distinct from others. MPP^{type2} cells and nuocytes were reported to respond to IL-25 alone, but NH cells do not respond to IL-25 without IL-2 but respond strongly to IL-33. NH cells are present in FALC but not in lymph nodes. Adoptive transfer experiments also demonstrated that NH cells do not migrate to the lymph nodes. Our results indicate that NH cells are distinct from nuocytes.

Poster Abstracts Session 1

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Expression of hsa-mir-15a, which is a candidate microRNA regulating VEGFA expression, is lower in CD4+ T cells in pediatric asthma patients

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Background: MicroRNAs are small non-coding RNAs that have been shown to regulate gene expression at the translational level via the RNA interference pathway. It has been shown that some microRNAs play roles in Th2-mediated pathology, although it is still unknown whether microRNAs are involved in asthma. Recently, Vascular endothelial growth factor (VEGF) play a role in the pathogenesis of asthma via induction of mucosal edema, narrows the airway diameter, and airway flow reduction. However,

it is unclear whether microRNAs are involved in VEGF regulation under asthmatic conditions.

Objective: To check the role of microRNAs in VEGF-A regulation under asthmatic conditions.

Methods: We analyzed the serum levels of VEGF-A in 20 pediatric asthma patients and 20 non-atopic healthy controls by ELISA. By real-time PCR, we also studied *VEGFA* mRNA expression and microRNAs expression in $CD4^+$ T cells from 25 pediatric asthma patients, 16 non-atopic pediatric controls, 10 atopic children who did not have asthma.

Results: Compared to non-atopic healthy controls, both the serum VEGF-A and expression of *VEGFA* mRNA in $CD4^+$ T cells were significantly higher in pediatric asthma patients ($p < 0.05$ in both). In addition, expression of *VEGFA* mRNA in pediatric asthma patients was also higher than that in atopic children who did not have asthma ($p < 0.05$). These data suggest that *VEGFA* expression in $CD4^+$ T cells may have a role in the pathogenesis of asthma, independently of atopic status.

We went on to analyze expression of candidate microRNAs regulating VEGFA. We found that expression of hsa-mir-15a, one of the candidate microRNAs, in $CD4^+$ cells was significantly lower in pediatric asthma patients, compared to non-atopic healthy controls or atopic controls, suggesting that high expression of hsa-mir-15a might post-transcriptionally down-regulate *VEGFA* mRNA expression in the pediatric asthma patients. Hsa-mir-15a levels were not correlated with serum levels of total IgE, suggesting hsa-mir-15a expression may be regulated independently of atopic status.

Conclusion: Expression of hsa-mir-15a, a candidate microRNA regulating VEGFA expression, is lower in $CD4^+$ T cells in pediatric asthma patients, independently of atopic status.

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Reduced diversity within the gut Bacteroidetes phylum in early infancy associates with delivery by caesarean section, atopic eczema development and delayed Th1 maturation

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Background: A low diversity of the gut microbiota in early childhood may be influenced by mode of delivery and could be more important than an altered prevalence of particular bacterial species for the increasing incidence of allergic disease. The advent of powerful, cultivation-free molecular methods makes it possible to characterize the total microbiome down to the genus level in large cohorts.

Methods: Microbial diversity and composition were analyzed with barcoded 16S rDNA 454-pyrosequencing in fecal samples at one week, one month, and 12 months of age in 20 infants with IgE-associated eczema and 20 infants without any allergic manifestation until 2 years of age. 12 infants were delivered by caesarean section. Circulating levels of Th1-associated CXCL10 and CXCL11 and Th2-associated CCL17 and CCL22 were assessed by Luminex at 6, 12 and 24 months.

Results: Vaginal delivery was associated with increased diversity within the Bacteroidetes phylum during the first month of life and more frequent detection of the major genus *Bacteroides*. At one month of age, infants with IgE-associated eczema had a lower diversity of the total microbiota (P=0.004) and a lower diversity of the bacterial phylum Bacteroidetes and the genus *Bacteroides* (P=0.02 and P=0.01) than healthy infants. Presence of the genus *Bacteroides* at one month was associated with high levels of the Th1-associated chemokines CXCL10 and CXCL11 during infancy. Low Th1- and high Th2-associated chemokine levels were observed in children developing allergic disease.

Conclusions: A reduced diversity within the Bacteroidetes phylum is observed in infants delivered by caesarean section and associates with an altered Th1/Th2 balance and atopic eczema development.

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Gene-environment interaction between TLR4/CD14/IL-13 polymorphism and bronchiolitis may influence the development of asthma in children

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Background: The aim of this study was to investigate the gene-environment interaction between *TLR4/CD14/IL-13* polymorphism and bronchiolitis in the development of asthma in children.

Method: A modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used to survey 1341 elementary school children and 919 nursery school children from Seoul, Korea. *TLR4*(rs1927911), *CD14*(rs2569190) and *IL-13*(rs20541) polymorphism were genotyped by TaqMan assay.

Results: The lifetime prevalence of asthma diagnosis in elementary school study and nursery school study were 9.6% and 8.7%, respectively.

Parental history of asthma (adjusted odds ratio [aOR], 2.56; 95% confidence interval [CI], 1.16–5.63, aOR, 3.60; 95% CI, 1.66–7.76, respectively), parental history of AR (aOR, 1.70; 95% CI, 1.04–2.78, aOR, 3.48; 95% CI, 1.95–6.19, respectively), and past history of bronchiolitis (aOR, 3.11; 95% CI, 1.84–5.24, aOR, 3.94; 95% CI, 2.27–6.84, respectively) were the independent risk factors for asthma diagnosis in elementary school children and nursery school children.

In elementary school children, Past history of bronchiolitis with CC type of *TLR4* polymorphism(aOR, 3.16; 95% CI, 1.15–8.71), CT+TT type of *TLR4* polymorphism(aOR, 4.23; 95% CI, 1.86–9.63), TT type of *CD14* polymorphism(aOR, 3.57; 95% CI, 1.34–9.51), CT+CC type of *CD14* polymorphism(aOR, 2.85; 95% CI, 1.32–6.16), GG type of *IL-13* polymorphism (aOR, 2.49; 95% CI, 1.02–6.10), AG+AA type of *IL-13* polymorphism(aOR, 3.21; 95% CI, 1.48–6.98) showed increase of risk, respectively.

In nursery school children, Past history of bronchiolitis with CC type of *TLR4* polymorphism(aOR, 4.23; 95% CI, 1.34–13.39), CT+TT type of *TLR4* polymorphism(aOR, 5.34; 95% CI, 2.00–14.29), TT type of *CD14* polymorphism(aOR, 7.22; 95% CI, 2.54-20.58), CT+CC type of *CD14* polymorphism(aOR, 4.49; 95% CI, 1.67–12.04), GG type of *IL-13* polymorphism (aOR, 4.09; 95% CI, 1.59–10.54), AG+AA type of *IL-13* polymorphism(aOR, 4.13; 95% CI, 1.64–10.38) showed increase of risk, respectively. But these results were unrelated to atopy and bronchial hyperresponsiveness.

Conclusions: Family history of asthma or AR and past history of bronchiolitis could be the independent risk factors for the development of asthma. Gene-environment interaction between asthma and bronchiolitis is modified by the *TLR4/CD14/IL-13* polymorphism.

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The evolution of IgE sensitization to Ascaris allergenic components in early infancy

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Background: The evolution of IgE antibody responses is influenced by the environment and the genetic background of the population. Allergen sensitization is directly influenced by exposure but also by other concurrent sources stimulating the immune system, such as *Ascaris lumbricoides*. It is expected that, under tropical conditions, sensitization to environmental allergens and clinical expression of allergy may be different to that observed in societies where parasitic diseases have been eradicated.

Methods: Based on the FRAAT birth cohort study in Cartagena, a Caribbean city of Colombia (Acevedo N, et al. *BMC Pulmonary Medicine* 2012, 12:13) we included 280 children and analyzed (ROC analysis, linear mix model, logistic regression, nonparametric correlations) the patterns of sensitization at four age ranges, S1 (6 - 11 months) to S4 (31 - 42 months) to Ascaris, *B. tropicalis* (Bt) and D. *pteronyssinus* (Dp) extracts (using ImmunoCap), and to Asc l 1 (ABA-1), Asc l 3 (Tropomyosin) and AscGST recombinant allergens (using ELISA), their relationships and effects on several outcomes.

Results: Overall, there was an increase in the frequency of IgE sensitization to all allergens during time ($p < 0.001$), however, that to AscGST tended to decline at S4. IgE levels to AscGST were the highest during S1 to S3, but at S4 IgE response to Asc l 1 was more intense. At S1 and S4 the frequencies of sensitization were: Asc l 1: 15.7 and 47%, Asc l 3: 19.2 and 36.8%, AscGST: 20.6 and 44.9%. Median total IgE at S1 was 28.1 KU/L (IQR 9.5-65.2) and at S4 185.5 KU/L (IQR 65.6-410.2), similar to that in mothers (194 KU/L, IQR: 88-629.5, $p = 0.6$). In contrast to serology for Ascaris extract, sensitization to Bt, Dp and recombinant allergens started as early as S1. At S4, 59% of children were sensitized to any recombinant allergen. Wheezing was associated with sensitization to Bt but not to Dp, Ascaris or the recombinant allergens. At S4 the prevalence of Bt sensitization in children was similar to that found in mothers. The cumulative prevalence of ascariasis (eggs in stool samples) was 10.5%. For detecting Ascaris infection, the 0.36 KU/L cut-off for Ascaris sensitization had less sensitivity (33.3%) than the 0.14 OD cut-off for Asc l 1 sensitization (67%). Regarding specificity, Ascaris serology (85%) is 1.4 times more specific than Asc l 1 determination (60%).

Conclusions: The prevalence of IgE sensitization in children living in a tropical environment is high. Sensitization starts very early for both Ascaris and dust mite allergenic sources and by three years of age reach adults' levels. Total IgE increases parallel to specific IgE levels. Sensitization to Bt was the only factor associated with wheezing. Sensitization to Asc l 1 is a good early marker of Ascaris infection.

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Bacteria-derived extracellular vesicles as an important causative agent for asthma and COPD

Young-Koo Jee

Many bacterial components in indoor dust can evoke inflammatory pulmonary diseases. Bacteria secrete nanometer-sized vesicles into the extracellular milieu, but it remains to be determined whether bacteria-derived extracellular vesicles in indoor dust are pathophysiologically related to inflammatory pulmonary diseases. Here, we evaluated whether extracellular vesicles (EV) in indoor air are causally related to the pathogenesis of asthma and/or emphysema. EV were prepared by sequential ultrafiltration and ultracentrifugation from indoor dust collected from a bed. Innate and adaptive immune responses were evaluated after once or 4 weeks airway exposure of EV, respectively. Vesicles 50-200 nm in diameter were present (102.5 microgram [based on protein concentration]/g dust) in indoor dust, and inhalation of 1 microgram of these vesicles for 4 weeks caused neutrophilic pulmonary inflammation. Additionally, polymyxin B (an antagonist of endotoxin, a cell wall component of Gram-negative bacteria) reversed the inflammation induced by the dust EV. Indoor dust harbors *Esherichia coli*-derived vesicles; airway exposure to the vesicles for 4 weeks induced neutrophilic inflammation and emphysema, which were partially eliminated by the absence of IFN-gamma or IL-17. Interestingly, serum dust EV-reactive IgG1 levels were significantly higher in atopic children with asthma than in atopic healthy children and those with rhinitis or dermatitis. Moreover, serum dust EV-reactive IgG1 levels were also elevated in adult asthma or COPD patients than in healthy controls. To sum up, EV in indoor dust, especially derived from Gram-negative bacteria, appear to be an important causative agent in the pathogenesis of asthma and/or emphysema.

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Utilization of nasal lavage exosomes as biomarkers in asthma with and without chronic rhinosinusitis – a proteomics approach

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Exosomes are nano-sized extracellular vesicles that recently have been described to have extensive regulatory functions in the body, both in immune function, organ homeostasis and cancer. Exosomes can deliver different functional RNA species from one cell to another,[1] and are also being investigated as biomarkers for primarily malignant disease.[2] Both the proteome and RNA content of exosomes depend on which cell produces these vesicles, as well as the biological status of the producing cell. Further, biomarkers to distinguish asthma endotypes, and asthma with or without major comorbidities, remains an unmet need in medicine, and could help the development of personalized medicine approaches. Nasal lavage exosomes may indeed be such a source.[3]

Nasal lavage fluid (NLF) was collected from subjects with and without asthma, as well as with and without chronic rhinosinusitis (CRS). Exosomes were purified using ultracentrifugation and filtration. Mass spectrometry, with exclusion lists, was first used to identify the proteome of exosomes from two pools of healthy individuals. A second quantitative analysis of the proteome was performed on exosomes pooled from NLF from four subsets of subjects; including healthy controls (n=14), CRS only (n=6), asthma (n=13) and asthma with CRS (n=15) (quantitative proteomics approach (TMT: tandem mass tags). Ingenuity Pathways Analysis (IPA) was used to analyze the composition and functions of the proteome.

604 proteins in the nasal lavage exosomes from healthy individuals. The use of repeated runs with exclusion lists, allowed a more detailed identification of the proteome, with several new proteins identified only in the second and third runs. A comparison with previously published exosomal proteins revealed 83 new exosomal proteins. Exploring differences in the exosomes from diseased individuals, 74 proteins were quantified in all. Mucins were increased in asthma with CRS, as well as in asthma without CRS, compared to healthy controls. Asthma with CRS and asthma expressed similar top networks in IPA, including functions such as "free radical scavenging". CRS only displayed a slightly different protein network in exosomes, containing functions such as "immune cell trafficking". The top biological function of asthma with CRS was "connective tissue disorder", while asthma alone was associated with "organismal injury", and CRS alone was associated with "inflammatory response".

This study shows that exclusion lists can be used to isolate otherwise undetectable exosomal proteins, and allowed for the identification of additional low abundance proteins. Furthermore, proteins associated with asthma, such as mucins, were observed in the exosomes of the asthma groups. The nasal exosome proteome of CRS was shown to be different to that of the asthma related diseases. This study is an initial step to develop nasal lavage exosomes as biomarkers in asthma with and without CRS, and may allow for the identification of novel mechanisms in different endotypes of both asthma and CRS, as well as their combinations.

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Silica crystals cause cellular damage in TLR3-activated human bronchial epithelial cells

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Background: Exposure to fine-particulate air pollution potentially causes serious health problems, including aggravation of asthma symptoms. Silica crystals (silica) are the main mineral component of yellow sand dust, and they activate the inflammasome to induce IL-1β secretion by macrophages.

Objective: The aim of this study was to evaluate the effects of silica on cultured normal human bronchial epithelial cells (NHBE).

Methods: NHBE were treated with TNF-α, LPS or Poly I:C in the presence and absence of various concentrations of silica (20-200 μg/ml). Cell supernatants were analyzed for IL-1β secretion by ELISA and Western blotting. Activation of caspase-1 was examined by Western blotting. Cell viability was assessed by MTT assay and by measuring release of LDH activity and HMGB1 protein.

Results: Poly I:C-, but not TNF-α- or LPS-, activated NHBE, showed silica dose-dependent elevation of the IL-1β protein level in the culture supernatant. However, simultaneous exposure to silica and Poly I:C did not induce caspase-1 activation of NHBE, and IL-1β protein detected in the supernatants was the precursor form (31 kDa), not the mature form (17 kDa). Furthermore, Poly I:C-activated NHBE showed significant decrease in MTT activity by simultaneous exposure to silica in a dose-dependent manner. Coincident with the decrease of cell viability, exposure to silica and Poly I:C strongly induced release of LDH activity and HMGB1 protein.

Conclusions: Unlike in macrophages, silica did not activate the inflammasome in NHBE. Instead, TLR3-activated NHBE were markedly damaged by exposure to silica. Our findings suggest

that inhalation of yellow sand dust during a viral infection may cause severe bronchial epithelial damage.

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The burden of asthma and perceptions of asthma control in South Korea: Results of the Asia-Pacific (AP) countries asthma insight and management (AIM) survey of patients

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Background: Despite the availability of effective medications and treatment guidelines, asthma's burden on patients remains high. The Asthma Insight and Management (AIM) Survey of patients from 8 Asia-Pacific countries and Hong Kong was performed in 2011. We report the asthma-related patient perceptions, behaviors, and presentation patterns, as well as recent trends in asthma management, in South Korea.

Methods: 80,761 households across Australia, China, Hong Kong, India, Malaysia, Singapore, South Korea, Taiwan, and Thailand were screened, and 3,630 households with an asthma patient ≥12 years old were identified. In South Korea, 9205 households were screened, and 400 asthma patients completed the survey. The survey consisted of 52 questions encompassing 8 health domains: general health, diagnosis/history, symptoms, exacerbations, patient burden, disease management, medications/treatments, and patient attitudes. The survey was conducted over the phone where randomization was viable (Australia, China, Hong Kong) or by random face-to-face interviews.

Results: There is a high degree of variation between countries in the Asia-Pacific region with respect to the overall disease burden imposed by asthma, including asthma control, exacerbation rates, disease management approaches, and resource utilization. Nearly one fourth of patients in South Korea (24%) reported having daytime symptoms every or most days in the past 4 weeks; 12% reported having nighttime symptoms every or most nights in the past 4 weeks. The major triggers of asthma episodes in South Korea are weather changes and viruses or colds. Although 27% of adults and adolescents with asthma in South Korea say they have been well or completely controlled in the past 4 weeks, only 8% would be classified as having controlled asthma according to GINA, while 55% would be classified as partially controlled and 37% as uncontrolled. Nearly one in two adults and adolescents with asthma in South Korea (47%) reported having episodes in the past year when their asthma symptoms were more frequent or severe than normal. On average, these exacerbations lasted 5 days and occurred 12 times per year for those patients who experienced them. Patients with asthma have a significant physical burden of disease, causing missed work and school days, lower productivity, and other activity limitations.

One in five asthma patients in South Korea (20%) have, at some time, felt their life was in danger during an acute episode.

Conclusions: In South Korea, asthma imposes a high societal burden in productivity loss as well as resource utilization arising from poor asthma control—despite the availability of effective asthma treatments and management guidelines.

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Prevalence of IgE-binding to Art v, Amb a and Hum s among pollen skin test positive patients from Northern China

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Objective: to investigate prevalence and cross-reactivity of specific IgE against weed pollen allergens in subjects with positive intradermal skin tests against pollen in Tangshan city, Northern China.

Method: Allergic patients visiting Department of Allergy in Tangshan Gongren Hospital during mid-June to mid-October 2011 underwent intradermal tests against 25 allergenic sources. Patients showing positive reaction to pollen (N= 144) were tested for specific IgE against Art v, Amb a, Hum s, Art v 1 and Amb a 1 by ADVIA Centaur.

Results: The specific IgE prevalence to Art v was 58% and 47% towards Art v 1. Hum s showed IgE prevalence of 41%. Amb a had 15% and Amb a 1 had 11% IgE-reactivity, respectively. Of the Art v IgE-positive patients, 76% were IgE-positive to Art v 1. Only 0.5% patients were IgE-positive to Art v 1 but negative to Art v. About 50% of the Hum s IgE positive patients were also Art v positive but there were no correlation between Hum s and Art v IgE. No Amb a IgE mono-sensitized patient was found and almost all Amb a IgE sensitive patients were also sensitized to Art v.

Conclusion: The prevalence and correlation of specific serum IgE suggests that pollens of Art v and Hum s are important and independent allergenic sources among patients in northern China. No patients with specific IgE only to Amb a could be detected and 90% of Amb a IgE positive patients had Art v specific IgE, suggesting that Amb a is a relatively less important source of primary sensitization among these patients.

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The development of atopic dermatitis according to age of onset and the association with early life exposures

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Background: Environmental factors may affect the development of atopic dermatitis and this was described to be already effective during pregnancy and in early life. An important early postnatal exposure is nutrition, though its association with allergic disease remains unclear.

Objective: To determine prospectively whether early postnatal exposures, such as the introduction to complementary food in the first year of life, are associated with the development of atopic dermatitis, taking into account the reverse causality.

Methods: 1041 children who participated in a birth cohort study, the Protection against Allergy-Study in Rural Environments were included in this study. Atopic dermatitis was defined by doctor diagnosis reported by the parents of children up to 4 years of age, by questionnaires, and/or with positive Scorad score from 1 year of age and according to the age of onset, within or after the first year of life. Feeding practices were reported by parents in monthly diaries between the 3rd and 12th month of life.

Results: The diversity of introduction of complementary food in the first year of life was associated with a reduction of the risk of having atopic dermatitis with onset after the first year of life (adjusted odds ratio for atopic dermatitis with each additional major food item introduced, 0.76; 95%CI, 0.65- 0.88). The introduction of yogurt in the first year of life also reduced the risk for atopic dermatitis (adjusted odds ratio, 0.41; 95%CI, 0.23- 0.73).

Conclusion: As early life exposure, the introduction of yogurt and the diversity of food introduced in the first year of life might have a protective effect against atopic dermatitis.

Poster Abstracts Session 2

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Differential impact of glutamate and kynurenines on CD4+ T cell subtype function

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Background: Through the rate-limiting enzyme, indoleamine 2,3 dioxygenase (IDO), oxidative catabolism of tryptophan generates kynurenines that are biologically active on neurons. We have previously shown that human and murine eosinophils constitutively express IDO, supernatants from which showed differential effects on Th1 and Th2 cell proliferation. Recent reports also showed positive correlations of IDO activity with Th2 responses. NMDA receptor binds components of kynurenines that are glutamate surrogates. Ca^{2+} flux through NMDA receptors is thought be critical in synaptic plasticity, however, prolonged and excessive activation of these receptors, is associated with neuronal death (excitotoxicity) in amyotrophic lateral sclerosis, HIV dementia and other neurodegenerative conditions. Although the impact of NMDA receptor activation is convincing, it remains confined to neuroscience. Current knowledge of NMDA receptors in immune regulation, including allergy and asthma, is very limited. Thus, we aimed in this study to investigate if glutamate and kynurenines influence T cell function.

Methods: Eosinophils, conventional $CD4^+$ and naïve $CD4^+$ T cells were separated from PBMC from consenting healthy donors, and differentiated into Th1 and Th2 cells using established methods. Expression of glutamate receptors and transporters was detected using RT-PCR, quantitative real-time PCR and flow cytometry. T cells proliferation, apoptosis and viability was detected by CFSE-, Annexin V-staining and MTT assay respectively. Ca^{2+} flux was measured using Fluo-3 and Fura Red.

Results: Detectable expression of NMDA receptors was found on $CD4^+$ T cells, but not eosinophils. $CD4^+$ T cells and its subtypes were stimulated by glutamate and kynurenines, which exerted apoptotic and anti-proliferative effects of NMDA receptors activation on Th1, but not Th2 cells. This was also true for Ca^{2+} flux. All these effects were significantly inhibited with the NMDA receptor specific competitive inhibitor, MK-801.

Conclusion: This study showed that, like neurons, $CD4^+$ T cells respond to glutamate and kynurenines through NMDA receptors. Our data suggested that NMDA receptors-dependent cell death mechanism may be a potential regulator of T cell balance by leading apoptosis in Th1 cells, which can result in Th2 polarization. These studies have major implications for allergy and asthma including potentially therapeutic strategies that target NMDA receptors on T cells.

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How splenic long-lived plasma cells impinge on humoral immunity

Jeehee Youn, Eunkyeong Jang, Wang-Sik Cho

Background: Long-lived plasma cells (LL-PCs) are crucial for persisting chronic inflammatory disorders, but how they impinge on the pathogenesis remains unclear. Previously we have found that $Foxp3^+$ regulatory T cell-depleted autoimmune mice, referred to as K/BxNsf, exhibited an abnormal accumulation of autoantigen-specific LL-PCs in their spleens but not in the bone marrow.

Methods: The phenotypic and functional features of splenic LL-PCs from K/BxNsf mice were compared to those of conventional short-lived plasma cells (SL-PCs) from their control littermates

Results: K/BxNsf LL-PCs were less susceptible to the cytotoxic action of cyclophosphamide and escaped from immune complex-mediated apoptosis by downregulating FcγRIIb. They expressed lower levels of costimulatory molecules and the intracellular machinery for antigen presentation than SL-PCs, which was associated with their reduced capacity of $CD4^+$ T cell priming. Most importantly, antigen-pulsed LL-PCs drove $CD4^+$ T cells to induce follicular helper T cell-specific factors such as BCL-6 and IL-21, while SL-PCs failed to do.

Conclusion: Thus, our results suggest that, unlike SL-PCs, LL-PCs abnormally accumulated in the spleen of autoimmune mice are not able to form a negative feedback loop with follicular helper T cells, thereby favoring the persistence of humoral autoimmunity.
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CD8 T cells: master regulators of the immune response in infection and inflammation

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CD8 T cells are best known for their ability to kill viral infected cells but recent research has suggested a much wider range of functions. CD8 T cells are the major source of the anti-viral cytokine interferon-gamma (IFN-γ). In this study we investigated the role of IFN-γ in the contraction of the immune response and lung pathology post influenza infection. IFN-$\gamma^{-/-}$ mice were infected with 5 plaque forming units (pfu) of influenza H1N1 (PR8) and sacrificed at different time points. Bronchoalveolar lavage (BAL) was collected and cells identified by flow cytometry. Eosinophils were identified as SINGLEC-F +ve and CD11c-ve and neutrophils as Ly6G+ve and CD11c-ve. BAL cytokines were determined by ELISA. Lung tissue was dissociated using liberase and eosinophils and neutrophils identified by flow cytometry. A higher percentage of influenza antigen-specific (NP_{366}) CD8 T cells from IFN-γ deficient mice expressed IL-7Ra (CD127) at day 8 (wild type, WT 5%; IFN-$\gamma^{-/-}$ 13% $p<0.01$). On reinfection with HK-x31 that contains the H3N2 surface hemagglutinin and neuraminidase molecules and the internal components of the PR8 virus (Kilbourne ED *Bull Wld Hlth Org* 1969; 14: 643-5) (Kind gift from Sivasankar Balasubramanian), IFN-$\gamma^{-/-}$ mice produced a greater lung CD8 T cell response (WT 80,000 and IFN-$\gamma^{-/-}$ 300,000 NP_{366} CD8 T cells $p< 0.01$). On day 8 post infection (p.i.) there were increased amounts of BAL IL-5 (WT 80, IFN-$\gamma^{-/-}$ 280 pg/ml $p<0.001$), Eotaxin (WT 100, IFN-$\gamma^{-/-}$ 220 pg/ml $p<0.01$) and IL-10 (WT 20, IFN-$\gamma^{-/-}$ 320 pg/ml $p<0.001$). On day 11 p.i. there were increased numbers of eosinophils (WT 10,000, IFN-$\gamma^{-/-}$ 175,000 cells/lung $p<0.001$), macrophages (WT 25,000 IFN-$\gamma^{-/-}$ 140,000 cells/lung $p<0.001$) but not neutrophils (WT <500, IFN-$\gamma^{-/-}$ <500 cells/lung). Comparable results were obtained in IFN-γ receptor deficient mice. These data indicate that IFN-γ plays an important part in the resolution of the CD8 T cell response in influenza and raises the possibility that compromised IFN-γ production may contribute to the production of airway T helper 2 cytokines and eosinophilic inflammation.

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Development of the innate immunity in childhood and the association with environmental exposures

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Background: The innate immune system constitutes the first contact to invading microbes and provides co-stimulatory molecules and cytokines to direct the adaptive immune response. It was suggested that the innate immune system is involved in the protective effect of exposures from farming environment on the development of allergic diseases.

We sought to determine the development of the innate immunity over time from birth to childhood and, its association with environmental factors.

Method: Children who participated in a birth cohort study, the Protection against Allergy-Study in Rural Environments (PASTURE) were included in this study. Gene expression of molecules from the innate immunity was assessed in blood leucocytes of samples from birth (n=943), 1 (n=779), 4.5 (n=683) and 6 (n=672) years of age by quantitative PCR. Environmental exposures were reported by the parents yearly by questionnaires.

Results: During childhood we observed a change of the expression of innate immunity genes. Within the first year of life we observed a significant increase of most of the gene expression of the innate immunity, followed by a slight decrease later in life. The pattern of the evolution of gene expression over time was similar among farmer and non-farmer and the farm-related exposures did not explain the increase of gene expression in the first year of life. However, we observed for some genes, especially the receptors of the innate immunity, an up-regulation with the farming status.

Conclusion: The expression of innate immunity genes changes, (1) with age, during childhood and (2) with environmental exposures.

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Contact dermatitis in psoriasis patients: a powerful model to analyze disease pathogenesis

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Background: Both eczema and psoriasis are inflammatory skin conditions defined by epithelial alterations and recruitment of immune cells, mostly lymphocytes, to the site of inflammation. While allergic contact dermatitis (ACD) is an immune reaction limited to the area of direct contact with generally non-harmful substances such as allergens or haptens, psoriasis is a systemic disease with abnormal keratinocytes that are less differentiated, more proliferating and more resistant to apoptosis than healthy keratinocytes. It is unknown whether contact dermatitis is altered under these conditions.

Method: We induced acute contact dermatitis reactions against the allergen Der p 1 (atopy patch test, APT; n=2) and against the hapten nickel (n=12) in sensitized psoriasis patients. Biopsies of acute eczematous reactions and fresh neighboring psoriasis plaques were investigated histologically, and T cells were isolated from lesional skin and subsequently characterized for cytokine secretion by ELISA and flow cytometry as well as for their specificity in co-culture experiments with autologous monocyte-derived dendritic cells.

Results: Clinically, all 14 patients developed typical eczematous lesions upon antigen provocation. However, the reaction was delayed compared to non-psoriatic individuals, reaching its peak after 7-10 days. Histological analysis revealed typical hallmarks for eczema in ACD and for psoriasis in psoriasis lesions, respectively. T cells isolated from APT reactions to Der p were mainly, but not exclusively, of the Th2 subset, while the parallel obtained psoriatic lesions of the same patients showed a Th1/Th17 dominated T

cellular infiltrate. Moreover, only in APT reactions Der p 1 specific T cells were found, and those were almost exclusively Th2 cells. Interestingly, *filaggrin* expression was lower in APT reactions than in psoriasis plaques, confirming a suppressive role for Th2 cytokines on *filaggrin* synthesis. In contrast to APT reactions, the T cellular infiltrate of ACD lesions to nickel was comparable to the one of psoriasis plaques with the exception that slightly more Th2 cells were present in ACD.

Conclusions: In summary, this study demonstrates that psoriasis patients develop typical, but probably delayed, contact dermatitis reactions. Thus, rather than genetically determined epithelial alterations, the antigen determines the pattern and clinical outcome of a cutaneous T cell immune reaction.

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Phospholipase A_2 of peroxiredoxin 6 has a critical role in tumor necrosis factor-induced apoptosis in human bronchial epithelial cells (BEAS2B)

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Background: Peroxiredoxin 6 (Prdx6) is a bifunctional enzyme with peroxidase and phospholipase A_2 (PLA_2) activities. Prdx6 is expressed in all major mammalian organs, with the greatest protein expression levels in the lung. Within the lung, especially high expression is observed in type 2 alveolar epithelial cells and bronchiolar Clara cells, indicating preferential roles for Prdx6 in lung-resident cells.

Objective: Although the cellular function of the peroxidase of Prdx6 has been well elucidated, the function of the PLA_2 of Prdx6 is largely unknown. Here, we report a novel function for the PLA_2 in regulating TNF-induced apoptosis through arachidonic acid (AA) release and interleukin-1β (IL-1β) production.

Methods and Results: Prdx6 knockdown ($Prdx6^{KD}$) in human bronchial epithelial cells (BEAS2B) shows severe decreases of peroxidase and PLA_2 activities. Surprisingly, $Prdx6^{KD}$ cells are markedly resistant to apoptosis induced by TNF-α in the presence of cycloheximide, but are highly sensitive to hydrogen peroxide-induced apoptosis. Furthermore, the release of AA and the production of IL-1β induced by proinflammatory stimuli, such as TNF-α, LPS, and poly I/C, are severely decreased in $Prdx6^{KD}$ cells. More interestingly, the restoration of Prdx6 expression with wild-type Prdx6, but not PLA_2-mutant Prdx6 (S32A), in $Prdx6^{KD}$ cells dramatically induces the recovery of TNF-induced apoptosis, AA release, and IL-1β production, indicating specific roles for the PLA_2 activity of Prdx6.

Conclusions: Our results provide new insights into the distinct roles of bifunctional Prdx6 with peroxidase and PLA_2 activities in oxidative stress-induced and TNF-induced apoptosis, respectively.

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Characterisation of grass pollen allergen-driven memory B cells in human patients with allergic respiratory disease

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Grass pollen allergens are major triggers of allergic respiratory disease. IgE is critical for triggering allergic reactions and perpetuating ongoing allergic inflammation. However, surprisingly little is understood about the qualities of allergen-specific B cells. Whether allergen-driven IgE-switched B cells develop via the traditional pathway of activation and maturation of adaptive B cells within germinal centers remains unclear.

B cell responses from blood of 13 patients with grass pollen allergy and 12 non-allergic healthy donors were examined by ELISA and flow cytometry. Peripheral blood mononuclear cells were isolated and stained with a cytoplasmic dye carboxyfluorescein diacetate succinimidylester (CFSE) and cultured for 5 days with Bahia grass pollen allergen extract, influenza antigen, phytohaemagglutinin or without stimulus. Cellular proliferation was measured by dilution of CFSE and the surface phenotype of allergen-driven B cells was assessed using multicolor flow cytometry.

Subjects with grass pollen allergy showed higher serum IgE reactivity with Bahia grass pollen than non-allergic controls ($p = 0.0001$). Both B (CD19+) and T (CD3+) cells from grass pollen allergic donors showed greater proliferation to grass pollen allergen than non-allergic donors ($p = 0.0031$, and $p = 0.0127$, respectively), whereas responses to a viral antigen did not differ between patients and controls. Allergen-driven B cells that entered into division early (CD19+ CD3- CFSElo) showed higher expression of the activation marker CD27 ($p = 0.0078$) and lower expression of molecules associated with B cell receptor complex; CD19 ($p = 0.0039$) and CD20 ($p = 0.0039$) than B cells that were slow to divide (CD19+ CD3- CFSEmid). Moreover, rapidly dividing allergen-driven B cells (CD19+ CFSElo CD27hi) showed higher expression of the plasmablast marker CD38 compared with B cells that were slow to respond to allergen (CD19+ CFSEmid CD27lo cells).

These data are consistent with rapid *in vitro* expansion of a population of grass pollen allergen-specific memory B cells in the blood of allergic patients that is absent in non-allergic control donors. We speculate that these circulating allergen-driven memory B cells may be precursors of IgE-switched B cells.

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Bronchial mucosal leukocytes in aspirin sensitive asthma show deficient expression of the PGE2 receptor EP2

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Background: Prostaglandin E2 is an important anti-inflammatory eicosanoid in asthma. The purpose of this study was to extend our previous observations implicating impaired leukocyte responsiveness to PGE2 as a pathogenetic mechanism in aspirin sensitive asthma, which might partly account for the observed acute exacerbation of asthma and rhinosinusitis in these patients on exposure to cyclooxygenase inhibitors.

Method: Immunohistochemistry was employed to enumerate inflammatory cells and their expression of the cysteinyl leukotriene receptors CysLT1 and CysLT2 and the PGE2 receptors EP1-4 I the bronchia mucosa of aspirin sensitive and tolerant asthmatics and controls (n=15 per group). Bronchoalveolar lavage (BAL) concentrations of PGE2 were measured by ELISA. The effects of PGE2 and EP receptor agonists on CD3/CD28-stiumlated cytokine production by blood mononuclear cells were measured by ELISA. Airway responsiveness to LTD4 *in vivo* was measured in all the asthmatics by bronchial challenge.

Result: Compared with tolerant asthmatics, aspirin sensitive asthmatics had reduced percentages of T cells, macrophages, mast cells and neutrophils expressing EP2. Both groups of asthmatics showed elevated bronchial reactivity to LTD4 but this did not correlate with mucosal expression of CysLT1 or CysLT2. BAL PGE2 concentrations were comparable in all groups. *In vitro*, PGE2 inhibited cytokine production by blood mononuclear cells via the EP2, but not the other PGE2 receptors.

Conclusions: Our data are consistent with the hypothesis that impaired, EP2-mediated leukocyte inhibition has a role in the pathogenesis of aspirin sensitive asthma. Our data are also consistent with the hypothesis that neither CysLT1 nor CysLT2 mediate bronchial responsiveness to cysteinyl leukotrienes ***in vivo***.

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Human milk oligosaccharides reduce respiratory viral infection and inflammation in human cells

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Rationale: Breast feeding is associated with a reduced incidence of respiratory infections compared to formula fed infants. Human milk oligosaccharides (HMOs) may significantly contribute to this protective effect. During feeding, HMOs bathe the laryngopharyngeal region at the entry to the upper respiratory tract, which would include epithelial cells as well as locally resident or transient immune cells. Therefore, the effects of HMO interaction with human respiratory epithelium and peripheral blood mononuclear cells during respiratory viral infection were investigated.

Methods: Human PBMCs (peripheral blood mononuclear cells), or human respiratory epithelial cell lines were treated ***in vitro*** with 4 HMOs individually and some in combination. Cells were then infected with respiratory syncytial virus (RSV) or influenza virus. Cytokine results were measured at 24 and 48 hours post-infection. Viral load was measured at 48 hours post infection.

Results: In epithelial cells, acidic or neutral human milk oligosaccharides (HMOs) significantly decreased production of RSV, while acidic HMOs decreased production of influenza. HMOs also significantly decreased production of pro-inflammatory cytokines (IL-6, IL-8 and TNF-alpha) in epithelial cells. In PBMCs, acidic HMOs had the most potent effect on decreasing IP-10, a clinical marker correlating to RSV severity. PBMC data also showed that acidic and neutral HMOs increased IL-10, an anti-inflammatory cytokine.

Conclusions: *In vitro* data show select HMOs dampen inflammatory cytokine expression and reduce viral load in human PBMC and respiratory epithelial cell lines exposed to RSV or influenza. HMOs are effective in PBMCs at human milk levels, and are effective in respiratory epithelia at levels lower than those found in human milk. These data indicate HMO have the potential to beneficially impact human respiratory health.

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Airway immunoexpression of CD206, a marker of alternatively activated macrophages, is increased in asthma and influenced by disease severity and inhalation allergen challenge

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Background: The Th2 cytokines, IL-4 and IL-13, have potentially important interactions with the innate immune response, in that they may promote the generation of alternatively activated macrophages (M2 phenotype). Such M2 macrophages have impaired phagocytic activity and exaggerated TNF-alpha generation, features more prominent in severe treatment-resistant asthma. This study has thus investigated the airway immunoexpression of the type 1 mannose receptor (CD206), an M2 phenotypic marker. Evaluation has been undertaken in endobronchial biopsies from volunteers with mild asthma, treatment-resistant severe asthma and healthy controls. The influence of repeated experimental allergen challenge has also been evaluated in non-steroid treated atopic asthmatics (n=12), and compared to the findings in a matched control group undergoing repeated methacholine challenge (n=12).

Methods: Endobronchial biopsies, processed into GMA resin, were obtained from 11 healthy controls (6F, mean age 21.7 yrs, mean FEV_1 97.5% predicted), 25 mild asthmatics (19F, mean age 24.7 yrs, mean FEV_1 98.3% predicted) and 14 severe asthmatics (9F, mean age 45.7 yrs, mean FEV_1 58.6 % predicted). Immunostaining was undertaken in tissue sections with a monoclonal antibody against CD206 (Abcam, Cambridge UK, 1:200 dilution) along with appropriate isotype and negative controls. Immunoreactive

positive cells were enumerated in the sub-mucosa by image analysis and related to tissue area, excluding glands and airway smooth muscle.

Results: There was significantly greater expression of CD206 in both mild asthma (p=0.002) and severe asthma (p<0.0001) than in the healthy controls, with the number of immunopositive CD206 cells being significantly greater in severe asthma than in mild asthma (p=0.001). Experimental allergen challenge significantly increased the number of sub-mucosal immunoreactive CD206 +ve cells per mm^2 from a mean of 6.4 to 17.8 whereas methacholine challenge was without effect (pre mean 6.5, post mean 7.6) The change with challenge was significantly greater (p=0.02) with allergen (11.4) than methacholine (1.1)

Conclusions: These findings are indicative of increased expression of alternatively activated macrophages within the airways in asthma and in particular severe asthma. Furthermore airway mucosal CD206 expression in asthma is influenced by immunological but not bronchoconstrictor responses to allergen. These findings have relevance to disease understanding, particularly in severe asthma.

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Chronic chlorine exposure aggravates allergic asthma via innate immune system in a murine model of asthma

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Background: Previous epidemiologic studies suggested that chronic exposure to chlorine products would be associated with development and aggravation of asthma. However, its exact pathomechanism was not clearly elucidated yet. This study was aimed to investigate the effect of chronic exposure to low dose chlorine gas vaporized from sodium hypochlorite (bleach) on airway inflammation and airway hyperresponsivenes.

Methods: Six week-old female BALB/c mice were grouped into the control group (PBS group), the hypochlorite group (PBS/Cl group), the ovalbumin (OVA) group, and the OVA/hypochlorite group (OVA/Cl group). Mice of the PBS/Cl group or OVA/Cl group were exposed to naturally vaporized gas from 5% sodium hypochlorite solution for 8 hours a day for 4 weeks. Mice were sensitized with OVA intraperitoneally on day 1 and 14 and challenged via intranasal route on day 28, 29, and 30. Bronchoalveolar lavage (BAL) fluid cytokine levels were measured using multiplex ELISA and mRNA expressions of cytokines, alarmins and inflammatory mediators were measured using real time RT-PCR in lung homogenates. In vitro TSLP and IL-33 expressions were examined in epithelial cell lines after low dose sodium hypochlorite exposure.

Results: Airway hyperresponsiveness and inflammation were not induced by repeated low dose sodium hypochlorite exposure alone but those were significantly augmented by sodium hypochlorite co-exposure in the OVA-sensitized and challenged mice. Proinflammatory cytokine, IL-1β level in BAL fluid was significantly enhanced in OVA/Cl group compared with OVA group. Th2 cytokines, IL-4 and IL-5 and epithelial cytokine, IL-33 expressions were also significantly increased in OVA/Cl group compared with OVA group. In vitro experiment, TSLP and IL-33 expressions were upregulated after low dose sodium hypochlorite exposure in bronchial epithelial cells.

Conclusions: Low dose repeated chronic exposure of chlorine gas vaporized from sodium hypochlorite aggravates allergic asthma via innate immune system activation and subsequent augmentation of Th2 inflammation.

Keywords: chlorine, hypochlorite, bronchial asthma, animal model

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Phytoncide inhibits asthmatic reaction in murine asthma model

Mi-Kyeong Kim

Phytoncides are antimicrobial allelochemic volatile organic compounds derived from plants. People commonly engage in so-called forest bathing to breathe in phytoncides emitted by plant and trees, in order to improve their health, especially atopics. We declared healthy control's PEFR improved more than 10% during forest breathing.. In this study, we tried to find whether Phytoncides can improve pulmonary function and also allergic brochial inflammation in murine asthma model. The effects of phytoncide were evaluated by antibodies such as OA-IgE, IgG1, and OA-IgG2, Penh(enhanced pause, OMP-3000) measured by methacholine. and cytokines IL-4 and IL-5, INF-gamma and IL-10 by RT-PCR.

We found only 1% of phytoncide improved methacholine-induced Penh and inhibited cellular infiltration into the bronchi. IL-4 and IL-5 by RT-PCR were decreased, but INF-gamma increased. Infiltrated cells changed to small lymphocytes only, whose total number was 1/200 of allergic mice model in BAL fluid. But whole phytocide killed mice. 5% phytoncide did not work on murine asthma model neither improving Penh nor inhibit cellular infiltration. Phytoncide inhalation improves asthma through the through the generation of INF-gamma. This will be the new therapeutic candidate of allergic disease leading to the tolerance.

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Allergic airway inflammation and airway remodeling are more severe in female mice

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Background: Epidemiological studies have already shown that females are dominant in terms of the sex ratio of adult asthma prevalence and severe asthma. It has also been reported that female mice are more susceptible to the development of allergic airway inflammation and airway hyperresponsiveness(AHR)

than male mice. However, there have been few reports of studies on sex difference in the pathogenesis of severe asthma, especially airway remodeling in an animal model. In this study, we investigated sex difference in formation of airway remodeling using a long-term OVA challenged asthma model.

Methods: Following ovalbumin (OVA)/alum intraperitoneal injection, male or female mice (BALB/c) were challenged with aerosolized 1% OVA on 3 days/week for 5 weeks, and we investigated the sex difference in AHR, airway inflammation, as well as airway remodeling. In addition, we measured the levels of growth factors and Th2 cytokines in bronchoalveolar lavage fluid (BAL fluid) of each mice.

Results: In OVA-sensitized and -challenged (OVA/OVA) female mice, AHR, the number of eosinophils and lymphocytes, as well as Th2 cytokines and growth factors in BAL fluid were increased compared with OVA/OVA male mice. In addition, the histological features of airway remodeling, including goblet cell hyperplasia, subepithelial fibrosis and myofibroblast hypertrophy, were also increased in OVA/OVA female mice. Moreover, serum total and OVA-specific IgE were significantly elevated in OVA/OVA female mice.

Conclusions: These results indicate that female mice are dominant in terms of forming airway remodeling as compared with male mice. The involvement of sex difference for sensitization and growth factor release in lung tissue based on inflammatory cell infiltration is indicated for the mechanism of sex difference of airway remodeling.

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Effect of NADPH oxidase 2 (Nox2) deficiency on allergic lung inflammation and differentiation of helper T cells

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Background: The nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has been originally identified in phagocytes, which kills engulfed pathogens by producing reactive oxygen species (ROS) and often referred to as phagocyte NADPH oxidase (PHOX). It has been also revealed that activated T cells produce ROS and ROS is an important second messenger for the functions of T cell effectors. In addition, ROS scavenge during T cell activation showed decreased effector functions. However, recent studies have shown that NADPH oxidase 2 (Nox2) deficient CD4 T cells contain increased IL-17 producing capability and Nox2 knock-out mice have a tendency an enhanced susceptibility to spontaneous arthritis with age.

Objective: To investigate whether Nox2 or ROS deficiency in T cells but not in phagocytes affect differentiation and function of CD4 T cells in T cell intrinsic manner. Specifically we adopt allergic asthma model to clarify the function of Th2 cells without Nox2 or ROS.

Methods: Asthma phenotypes were analyzed in fungal protease allergen induced experimental asthma. To understand underline mechanisms of exaggerated effector T cell functions, we investigated the degree of T cell activation (status of hyper-activation), levels of activation induced cell death (AICD), and differentiation of regulatory T (Treg) cells with Nox2 deficient and wild type CD4 T cells.

Results: All experimental asthma phenotypes including airway hyper-responsiveness, lung inflammation along with eosinophilia, and mucus production were increased in Nox2 deficient mice. Nox2 deficient T cells also showed enhanced hyper-activation, reduced AICD, and decreased Treg cell differentiation.

Conclusions: Our results indicate that Nox2/ROS deficiency showed exaggerated experimental asthma, which was caused by enhanced function of Th2 cells.

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Molecular allergy diagnostics in allergic rhinoconjunctivits to grass pollen – a prerequisite for component-resolved specific immunotherapy

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Background: In a recent study molecular allergy diagnostics have been used to investigate IgE sensitization profiles to Phleum pratense in 176 children with allergic rhinitis and/or asthma from Rome/Italy. When these profiles were compared with a mixture of recombinant allergenic molecules previously proposed for component-resolved specific immunotherapy (SIT) [Tripodi et al. J Allergy Clin Immunol 2012 Mar;129(3):834-839], sensitization profiles were highly heterogeneous and matched this experimental SIT preparations profile in only 4% of cases while the remaining patients were classified in four mismatch categories.

Applying this concept to an adult population with grass pollen allergy, we aimed to correlate sensitization profiles to the allergens proposed for SIT.

Methods: 101 patients from Munich/Germany with allergic rhinoconjunctivitis (median age: 27 years; range: 18–64; females: 58%) were included in the study. 96% of the patients showed a positive reaction in the nasal and 57% in the conjunctival provocation test. All patients were sensitized to Phleum pratense and all sera were tested for IgE to the following allergens (cutoff, 0.10 kU/L): rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5b, rPhl p 6, rPhl p 7, rPhl p 11, and Phl p 12 (ImmunoCAP, Phadia, now Thermo Fisher Scientific, Uppsala, Sweden).

Results: By grouping patients according to sensitization profiles and subsequent matching to the experimental SIT preparation containing Phl p 1, Phl p 2, Phl p 5, and Phl p 6, the SIT preparations profile matched none of the 101 adult patients which were grouped in the three following mismatch categories: (1) Underpowered (= less allergens than sensitized to) (63.3%), (2) underpowered/overpowered (= more allergens than sensitized to) (33.7%), and (3) unrelated immunization (3%).

Conclusion: There is a substantial heterogeneity in IgE sensitization profiles to Phleum pratense in the adult patients investigated.

The degree of mismatch with the molecular profil proposed for SIT exceeds even the pediatric population cited above. Molecular allergy diagnostics is a prerequisite for component-resolved SIT both on the individual patient level as well as in epidemiological studies aiming to obtain knowledge on sensitization profiles which in turn should guide the composition of suitable SIT preparations.

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Long-term exposure to IFN-gamma enhances Th2 cytokine-induced gene expression in conjunctival fibroblast

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Background: Multiple cytokines usually co-exist in a tissue site, and they interact with each other, sometimes additively or synergistically, and sometimes suppressively. High concentrations of IFN-gamma, a well-known Th1 cytokine, are often detected in the tears of patients with severe allergic conjunctivitis. IFN-gamma reportedly suppresses Th2 type immune responses in the sensitization phase, but its role in the effector phase remains largely unknown. In the present study, we investigated the effects of IFN-gamma on the Th2 cytokine-induced gene expression profiles of human conjunctival fibroblasts.

Methods: Primary fibroblasts were obtained from non-allergic human conjunctival specimens and then cultured with IFN-gamma (10 ng/ml) for either 6 hours or 14 days. The cells were then washed, followed by stimulation with IL-4 (10 ng/ml) for 6 hours. Their mRNA expression profiles were determined using microarrays and qPCR.

Results: Fibroblasts treated with IFN-gamma for 6 hours showed significantly reduced IL-4-induced CCL11 expression compared with the untreated control fibroblasts. In sharp contrast, that expression was significantly enhanced when the cell treatment with IFN-gamma lasted 14 days. Further time-kinetic analysis found that less than 24 hours' treatment with IFN-gamma reduced Th2 cytokine-induced gene expression, whereas more than 72 hours' treatment enhanced them in a time-dependent manner.

Conclusion: Our results suggest that long-term exposure to IFN-gamma may not reduce, but actually enhance, allergic inflammatory reactions in the eyes, presumably through induction of some epigenetic changes in conjunctival fibroblasts.

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Allergen–specific T and B cell proliferation determined in pollen allergic patients by a CFSE dilution based assay

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Background: In past years alternative methods to radioactive measurements for cellular proliferation, such as assessment of dilution of the dye 5, 6-carboxy-fluorescein diacetate succinimidyl ester (CFSE) in the flow cytometer, have been established. The latter method has one major advantage: It allows not only for measurement of proliferation in total peripheral blood mononuclear cells (PBMCs) but also in subpopulations e.g. T cells or B cells by multicolour flow cytometry. In the present study we sought to establish a CFSE dilution based assay to determine which cell types contribute to a proliferative response to allergen *in vitro*.

Method: To that aim we isolated PBMCs from birch and grass pollen allergic patients and labelled them with CFSE. Cells were cultured in the presence or absence of highly purified recombinant allergens (rBet v 1, rPhl p 5) for one week whereupon proliferation was measured by flow cytometry.

Results: T cell proliferation upon allergen stimulation was observed in PBMC cultures of all patients over a wide range of allergen concentrations using co-staining for CFSE and the pan T cell marker CD3. More importantly co-staining for the B cell marker CD20 revealed, that also a sub-fraction of B cells proliferated in response to allergen stimulation.

Conclusions: Thus we demonstrated that in PBMC cultures of allergic patients both B and T cells proliferate specifically in response to allergen stimulation *in vitro*. Since each of the tested patients mounted allergen-specific antibody responses it is possible that the proliferating B cell sub-fractions contained allergen-specific B cells. The fact that in PBMC cultures of allergic patients not only T cells proliferate in response to allergen should be borne in mind when interpreting classical proliferation assays such as ^{3}H thymidine incorporation.

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Antigen-specific Th2-type immune responses certainly underlie the pathogenesis of non-IgE-mediated gastrointestinal food allergy

Hideaki Morita, Ichiro Nomura, Akio Matsuda, Kenji Matsumoto, Hirohisa Saito

Background: Most food allergies are IgE-mediated, but some patients—especially infants and young children—develop gastrointestinal symptoms several hours after ingestion of an offending food. Many such patients have no detectable IgE antibody to the offending food. Therefore, such gastrointestinal food allergy (GI allergy) is thought to be a non-IgE-mediated, cell-mediated disease. However, the precise pathogenesis of non-IgE-mediated GI allergy remains uncertain except for the fundamental role of TNF. The antigen-specific lymphocyte stimulation test (ALST) is a classic method for investigating antigen-specific T-cell responses, and it is thus suitable for investigating such responses in patients with GI allergy. However, the usefulness of ALST in patients with GI allergy is controversial, and we hypothesized that inconsistent results have been due to lipopolysaccharide (LPS) contamination of the antigen preparations. In this study, in order to clarify what types of antigen-specific immune responses are induced in patients with GI allergy, we determined the cytokine secretion profiles of peripheral blood mononuclear cells (PBMCs) from GI allergy patients stimulated with LPS-depleted milk protein preparations.

Methods: PBMCs from 65 patients with GI allergy, 12 patients with IgE-mediated cow's milk allergy (IgE-CMA) and 16 normal

infants, and cord blood mononuclear cells from 10 normal infants were cultured in the presence and absence of LPS-depleted milk proteins. Lympho-proliferative responses were assessed by ^{3}H-thymidine-uptake on day 5, and the cytokine secretion profiles in the supernatants were measured by Luminex multiplex systems on day 6.

Results: Obvious antigen-specific proliferations were found in 49 of 65 GI allergy, 11 of 12 IgE-CMA. Unlike in IgE-CMA, predominantly TNF-α was produced by PBMCs from patients with GI allergy ($P<0.01$). In contrast, Th2 cytokines—including IL-3, IL-5 and IL-13—were significantly produced by PBMCs from patients with GI allergy ($P<0.05$) and IgE-CMA. However, the concentrations of these Th2 cytokines tended to be higher in patients with GI allergy than in IgE-CMA.

Conclusion: Our results suggest that antigen-specific Th2-type immune responses underlie the pathogenesis of both GI allergy and IgE-mediated food allergy.

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The prevalence of gastroesophageal reflux disease in chronic unexplained cough

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Background: The common co-existence of cough and gastroesophageal reflux disease (GERD) is well established. Several respiratory guidelines for the management of chronic unexplained cough in adults advocate empirical treatment of GERD. In contrast, guidelines from some gastroenterological societies conclude that cough is unlikely to be related to GERD in the absence of heartburn or acid regurgitation. This study was performed to assess the prevalence of GERD in adults with chronic unknown cough.

Methods: Adult patients with unexplained cough more than 8 weeks were prospectively enrolled from January 2007 to December 2011 and underwent 24-hour impedance-pH monitoring of esophagus.

Results: The prevalence of GERD in chronic unexplained cough, as evidenced by an abnormal impedance or pH profile, was 46.3% (68 of 147 patients). Among 49 patients who were given anti-reflux medication for at least 3 months, 39 patients (79.6%) achieved total or near-total elimination of cough.

Conclusion: GERD, which is readily detected by 24-hour impedance-pH monitoring, is a common cause of chronic unexplained cough and can be successfully managed with anti-reflux therapy.

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The IL-1alpha/periostin/IL-6 axis contributes to the keratinocyte proliferation and differentiation in atopic dermatitis

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Background: Acanthosis (epidermal thickness) is a histological feature commonly observed in chronic inflammatory skin diseases such as atopic dermatitis. We previously found that periostin, an extracellular matrix protein produced by fibroblasts, is overexpressed in the dermis of atopic dermatitis patients, and its deficiency in mice led to attenuated epidermal thickness, as compared to wild-type mice, in a mite allergen-induced dermatitis model. However, it still remains obscure how periostin contributes to the epidermal thickness in the context of fibroblast-keratinocyte interaction.

Methods: To investigate the fibroblast-keratinocyte interaction, we used a three-dimensional organotypic co-culture system using keratinocytes and fibroblast as a model of skin tissue. We used either wild-type or periostin-deficient fibroblasts in this system.

Results: Wild-type fibroblasts efficiently supported keratinocyte proliferation in the three-dimensional organotypic co-culture system, whereas periostin-deficient fibroblasts did not. A profiling analysis using an array of antibodies against soluble factors showed that the expression of interleukin (IL)-6 was markedly impaired with periostin-null fibroblasts. Blocking antibodies against IL-6 strongly inhibited keratinocyte proliferation with wild-type fibroblasts. Conversely, addition of recombinant IL-6 dramatically promoted keratinocyte proliferation with periostin-deficient fibroblasts. We furthermore found that IL-1α secreted from keratinocytes acted on fibroblasts together with periostin to strongly induce the production of IL-6. Accordingly, blocking antibodies against IL-1α strongly inhibited keratinocyte proliferation with wild-type fibroblasts.

Conclusions: We propose that the IL-1α/periostin/IL-6 axis consists of a critical component in the epidermal thickness of atopic dermatitis. Excessive production of periostin, which is induced by the Th2 cytokines IL-4 and IL-13, may lead to the high IL-6 production by dermal fibroblasts and contribute to the epidermal thickness in atopic dermatitis.

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Host-pathogen interactions via bacteria-derived extracellular vesicles

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Background: Communication between cells and environment is an essential process in living organisms. The secretion of extracellular vesicles is a universal cellular process occurring from simple organisms to complex multicellular organisms. Throughout evolution, both prokaryotic and eukaryotic cells have adapted to

manipulate extracellular vesicles for intercellular communication. Extracellular vesicles, otherwise known as membrane vesicles, are spherical bilayered phospholipids of 20-200 nm in diameter produced ubiquitously from all Gram-negative bacteria and some Gram-positive bacteria investigated to date. The bacteria-derived extracellular vesicles are composed of membrane proteins, lipopolysaccharides, lipids, periplasmic and cytosolic proteins, DNA, RNA, and other factors associated with virulence. Although recent progress in this area has revealed that bacteria-derived extracellular vesicles play multiple roles in intercellular and interspecies communication, the pathophysiological role(s) and the mechanism(s) in inflammatory diseases are not understood.

Method: We evaluated the effect of bacteria-derived extracellular vesicles on systemic inflammation, lung inflammation and endothelial cell activation using *in vivo* and *in vitro* models.

Results: Intraperitoneal injection of bacteria-derived extracellular vesicles induced lethality via activation of systemic inflammation characterized by piloerection, eye exudates, hypothermia, tachypnea, leukopenia, disseminated intravascular coagulation, dysfunction of the lungs, hypotension, and systemic induction of inflammatory cytokines, chemokines, and endothelial cell adhesion molecules. We further showed that bacteria-derived extracellular vesicles act as more powerful systemic inflammatory response syndrome inducers than lipopolysaccharide alone, and that both vesicular lipopolysaccharide and other vesicular components including proteins should be crucial in the pathogenesis of extracellular vesicles-mediated inflammation.

Conclusion: Our study revealed a previously unidentified causative microbial signal in the pathogenesis of inflammation-related diseases, suggesting bacteria-derived extracellular vesicles as new therapeutic targets to prevent and/or treat inflammatory diseases caused by bacterial infection.

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Vitamin D levels and allergic diseases in Korean adults: The 2010 Korean National Health and Nutrition Examination Survey

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Background: Previous studies suggested that vitamin D has various roles on the immune response and allergic reactions. However, the effects of vitamin D deficiency on allergic disease have not been well determined.

Objectives: To investigate the influence of vitamin D level on allergic diseases and allergen sensitizations in adults.

Methods: We analyzed the association between serum 25-hydroxyvitamin D levels and asthma, atopic dermatitis and sensitization to three common aeroallergens from the data of the Korean National Health and Nutrition Examination Survey (KNHANES) in 2010. The current and past history, physician diagnosis of asthma and atopic dermatitis were surveyed by a questionnaire and sensitizations to *Dermatophagoides farinae*, cockroach and dog were evaluated by measuring serum levels of specific IgE. Allergic diseases and sensitization status were compared among the three ranks of serum 25-hydroxyvitamin D levels; deficiency (<15 ng/mL), insufficiency (15-29 ng/mL) and sufficiency (≥30 ng/mL).

Results: In 6,815 enrolled subjects, the mean level of vitamin D was 17.6 ng/mL. Although there was no significant difference in current presence of atopic dermatitis or asthma among the three groups of the vitamin D levels, subjects with lower vitamin D level had higher incidence of atopic dermatitis over a lifetime than those with sufficient vitamin D levels [hazard ratio (HR)=4.20, 95%CI 1.20-14.63, for vitamin D insufficiency; HR=5.04, 95%CI 1.42-17.82, for vitamin D deficiency] after adjusting age, sex and body mass index. Allergic sensitizations to any of three common allergens were not significantly different among the three ranks.

Conclusion: Lower levels of serum vitamin D were significantly related with the high prevalence of atopic dermatitis over a lifetime in adult population, while there was no association with asthma or atopy. These findings suggest that vitamin D deficiency has a significant effect on the development of atopic dermatitis.

Keywords: Vitamin D, atopic dermatitis, asthma, atopy, Korean National Health and Nutrition Examination Survey

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Investigation of the mechanisms of delay in anaphylactic and urticarial responses to red meat in patients with IgE antibodies to alpha-gal

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In 2008, we established that hypersensitivity reactions to the monoclonal antibody (mAb) cetuximab were causally related to pre-existing IgE antibodies to the glycosylation on this mAb (Chung et al, NEJM 2008). At the same time, we became aware of patients presenting with a novel form of food allergy, delayed anaphylaxis to red meat (Commins et al, JACI 2009 and AJRCCM 2012). This syndrome is also restricted to individuals who have IgE antibodies to alpha-gal, and our recent results have shown that tick bites are the primary cause of this form of sensitization (Commins et al, JACI 2011). The studies presented here are focused on understanding the delay between eating red meat and the onset of symptoms. Ten allergic patients with a history of urticarial reactions were challenged with red meat and monitored for the development of symptoms and visible skin reactions over six hours. Seven out of ten subjects developed pruritis and hives, with or without cramping. These subjects also had upregulation of CD63 on their basophils at the same time (i.e., 4-6 hours after eating meat). In two cases, symptoms were severe and one case has a marked rise in tryptase during the challenge. Ten controls were also challenged, and none of them developed either symptoms or hives over six hours. However, five of the controls

showed significant upregulation of basophil CD63 starting four hours after challenge. We have now shown that histamine increases in the circulation, both in allergic individuals and controls, and that this occurs in parallel with changes in CD63 expression. The results confirm that CD63 is a marker of basophil mediator release *in vivo*. In addition, the timing of the basophil changes is a reliable guide to the timing of the arrival of relevant antigen into the circulation. However, it is equally clear that basophil responses are not a model for the mechanisms of urticaria/anaphylaxis. Our present data is best explained if absorption of alpha-gal on glycolipid particles takes four to six hours before it is in a form that is optimal for triggering mast cells in the skin or other tissues.

Oral Abstracts Session 3

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The impact of allergenic substances on epithelial barrier function in severe asthma

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Background: Exposure and sensitization to a range of allergenic substances have been associated with increased risk of asthma, as well as asthma exacerbations. Among the first cells to encounter inhaled allergens are epithelial cells at the airway mucosal surface. Since the bronchial epithelial barrier has previously been reported to be disrupted in asthma (Xiao et al J Allergy Clin Immunol 2011; 128:549-56), we hypothesized that pollen and fungal-derived substances may differentially affect epithelial barrier function and inflammatory responses of differentiated bronchial epithelial cells derived from severely asthmatic donors as compared with non-asthmatic controls.

Methods: Air-liquid interface (ALI) bronchial epithelial cultures from non-asthmatic or severe asthmatic donors were challenged apically with extracts of *Phleum pratense* (Timothy grass) pollen or *Alternaria alternata* spores; changes in inflammatory cytokine release, transepithelial electrical resistance (TER) and tight junction proteins were measured. Protease activity was characterised using a fluorometric assay.

Results: Using the fluorometric assay, no significant protease activity was detected in the pollen extracts, however *Alternaria* extracts contained predominantly serine protease activity that could be inhibited by heating. Following exposure of ALI cultures to grass pollen extract, the integrity of the epithelial barrier was not affected as monitored by measuring the transepithelial resistance and immunofluorescence staining of tight junction proteins. Although polarized cultures of undifferentiated BECs were sensitive to protease-mediated disruption of the epithelial barrier by *Alternaria* extracts, fully differentiated ALI cultures from non-asthmatic donors were completely protected against the barrier weakening properties of *Alternaria*. In contrast, ALI cultures from severely asthmatic donors were susceptible to barrier disruption by enzymatically active extracts of *Alternaria*. Even though the epithelial physical barrier was intact following pollen exposure, the immunological barrier became activated, as evidenced by changes in polarised mediator release. CXCL8/IL-8 showed the greatest increase in response to pollen exposure with preferential release to the apical compartment; this was mediated by activation of the ERK1/2 and p38 MAPK pathways. *Alternaria* extracts also stimulated release of IL-8, although in this case, basolateral IL-8 release was more responsive than apical release and this was reduced after heat inactivation of the extract. ALI cultures from severely asthmatic donors exhibited a significantly blunted IL-8 response to *Alternaria* relative to those from healthy donors.

Conclusions: Complex environmental stimuli like pollen and fungal spores have differential effects on the epithelial barrier, and differences in susceptibility of the physical barrier are evident in severe asthma. In response to environmental stimuli, the epithelial barrier also selectively modulates vectorial release of mediators that have the potential to promote migration and activation of inflammatory cells. These innate responses may differentially contribute to mucosal immunity depending on whether the epithelial barrier is compromised.

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Role of bronchial epithelial cells as a source of TF production and regulator of angiogenesis in asthma

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Background: Angiogenesis is an important facet of airway remodeling. It leads to an increase in the number and size of sub-epithelial blood vessels, resulting in thickening of the airway wall. A growing amount of evidence suggests that high levels of angiogenic mediators and increased microvessels are found in the airway of patients with asthma. Therefore, understanding the source and regulation of pro- and anti-angiogenic factors in the airway is a key unanswered question in asthma.

Method: Normal human bronchial epithelial cells (NHBECs) grown in air-liquid interface (ALI) culture were subjected to 30 cmH_2O compressive stress in vitro; this is equivalent to that which such cells would experience in situ during asthmatic bronchoconstriction. Conditioned media (CM) and cell lysates were collected from control and compressed cells. TF mRNA expression and secreted and intracellular proteins were analyzed by quantitative RT-PCR or ELISA and Western blot, respectively. Exosomes were isolated by ultracentrifugation and detected by transmission electron microscopy. The angiogenic potential of CM and bronchoalveolar lavage fluid (BALF) was determined using spheroid sprouting and murine aortic ring assays. Immunohistochemical staining was performed to identify epithelial expression of TF in asthma.

Results: Compressive stress applied to NHBECs increased TF mRNA expression and intracellular TF protein by 2.2-fold and 1.6-fold, respectively and provoked TF secretion over 50-fold compared to amount secreted in control cells. TF contained in exosomes was secreted in response to compressive stress in a PKC-dependent manner. The net effect of CM collected from compressed NHBECs was promotion of angiogenesis.

We found a higher concentration of TF in BALF from asthmatic than that from normal volunteers; much of this TF traveled with markers for exosomes isolated from asthmatic BALF. Furthermore, higher epithelial TF expression was detected in sections of endobronchial biopsies from asthma.

Conclusions: Our data showed that mechanically-stressed bronchial epithelial cells are an important source of secreted TF, and implicate exosomes as a key carrier of TF signals both *in vitro* and *in vivo*. These data highlight the potential role of epithelial secretion containing TF in promotion of angiogenesis in asthma.

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Distinct effects of Th1, Th2, Th17 and Treg cell subsets on regulation of bronchial epithelial tight junctions

Cezmi Ali Akdis

Background: Tight junctions (TJ) form homodimeric cell-cell contacts and control cellular integrity and paracellular flow of molecules and cells, and represent an essential component of epithelial cell barrier. Their regulation by T cell subsets in chronic allergic inflammation has not been previously reported.

Methods: We investigated the expression and regulation of epithelial tight junctions by fluidic card PCR, trans epithelial resistance, paracellular flux analyses of human primary epithelial cells, air liquid interfaces and cocultures with *in vitro* differentiated and primary T cell subsets and their prototype cytokines. Their *in vivo* regulation has been investigated in mouse model of asthma and direct analysis of human biopsies.

Results: Detailed expression analyses of 62 different junctional molecules demonstrated distinct and overlapping patterns of tight junctions and other cell-cell adhesion molecules in skin, sinus, tonsil and lung epithelia, which implicate a highly regulated and complex pattern. Each cell type shows its own, unique expression profile reflecting their specialization related to their function within the organism. T cells subsets show distinct regulation of epithelial tight junctions. Th1 cells and their cytokines particularly IFN-γ and TNF-α open epithelial tight junctions and significantly disrupt epithelial integrity. Th2 cells co-cultured with air liquid interface cultures of human primary bronchial epithelium significantly decreased transepithelial resistance and increased paracellular flux particularly mediated by IL-4 and IL-13, but not IL-25 and IL-33. CD4+ Foxp3+ Treg cells also open tight junctions as demonstrated by decreased transepithelial resistance and confocal microscopy. Their intreatreacheal administration decreased peribronchial inflammation, prevented the down-regulation of the TJ proteins and lead to a strong regeneration of epithelial integrity including TJ expression in mouse model of asthma. Th17 cells and IL-17 did not show any effect on epithelial cell integrity and regulation of tight junctions.

Conclusion: These data demonstrate a complex regulation of epithelial tight junctions and bronchial epithelium integrity by different T cell subsets and suggest a novel approach for the treatment of asthma.

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The role of caveolin-1 in epithelial barrier dysfunction in asthma

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Background: In asthma, mucosal barrier function is often compromised, with disrupted expression of the adhesion molecule, E-cadherin. Recent data suggest that E-cadherin contributes to the structural and immunological function of airway epithelium, through the regulation of epithelial junctions, proliferation, differentiation, and production of growth factors and pro-inflammatory mediators that can modulate the immune response. We hypothesized that loss of caveolin-1, the main component of caveolae, known to stabilize E-cadherin at the plasma membrane, contributes to epithelial barrier dysfunction in asthma.

Methods: Airway tissue and Air-liquid interface (ALI) culture sections from asthmatic (n = 9) and healthy donors (n = 9) were analyzed by immunofluorescence and western for Caveolin-1, E-cadherin, and b-catenin expression. Epithelial barrier function was determined using the Electric Cell-substrate Impedance Sensing system (Applied Biophysics, NY) and culture supernatants were analyzed for TSLP and IL-33 using commercial ELISAs. Caveolin-1 specific siRNA knock-down experiments were analyzed in the human bronchial epithelial cell line, 16HBE.

Results: We demonstrate that asthmatic epithelium *in vivo* displays reduced levels of caveolin-1 (P=0.045), accompanied by lower junctional expression of E-cadherin (P=0.019) and b-catenin (P=0.037) compared to non-asthmatic epithelium. This disrupted membrane expression of E-cadherin and caveolin-1 is paralleled by lower resistance in epithelial cells derived from asthma patients when compared to non-asthmatic epithelium (P=0.04). When asthmatic derived epithelial cells are cultured at ALI for 28 days they maintain down-regulated expression of Caveolin-1 (P=0.028) and E-cadherin (P=0.044) which is associated with elevated release of the Th2 promoting cytokines TSLP (P=0.021) and IL-33 (P=0.028), compared to non-asthmatic derived ALI cultures. Importantly, we show that in 16HBE cells, the down-regulation of caveolin-1 with targeted siRNA results in delocalization of E-cadherin and reduced barrier function (P=0.037) due to loss of E-cadherin-mediated cell-cell contacts.

Conclusions: This parallel *ex vivo* and *in vitro* study of the asthmatic epithelium demonstrates that caveolin-1 plays a crucial role in the maintenance of epithelial barrier function in asthma.

Importantly, this loss of barrier function due to down-regulation of caveolin-1 is associated with promotion of a Th2 pro-inflammatory phenotype. Targeting caveolin-1 may constitute a new therapeutic strategy to improve of mucosal barrier function in asthma.

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A novel aspect of autoallergy: Dichotomic IgE-autoreactivity-pattern of skin/gut versus airway epithelial cells

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Background: Traditionally, Th2-mediated allergies and Th1/Th17-biased autoimmunity (e.g. Multiple sclerosis) were regarded mutually exclusive, due to their opposing cytokine profiles. This dogma was challenged by the observation of autoreactive IgE (auto-IgE) and CD4 T cells directed against skin-derived proteins in atopic eczema (AE). Because auto-IgE was mainly reported in adults with severe and chronic disease, a role for IgE-mediated autoinflammation was assumed in chronic AE, even in the absence of exogenous allergens. Until today, it has never been addressed whether auto-IgE is a pathogenic driver for disease chronicity or just an immunological epiphenomenon. Moreover, it remains unclear whether auto-IgE targets the skin exclusively and is present only in chronic adult AE, or whether it indicates the loss of immune-tolerance already in childhood AE.

Method: We established a high-throughput immunoassay to analyze IgE-autoreactivity against protein extracts from primary keratinocytes, intestinal- (FHs74 Int.) and airway epithelial (NHBE) cells in sera from patient samples (106 AE-patients, 106 atopic patients; 10 mo.–70 yrs.).

Results: 25% of all AE-patients showed IgE-autoreactivity against control-keratinocytes, 29% against keratinocytes from AE-pts., 33% against intestinal cells and 25 % against airway epithelium. The prevalence and intensity of IgE-autoreactivity was strongly associated with age, since the highest prevalence (50%) and highest levels of autoreactivity were detected in young children (1-10y.). An analysis of each patient's individual organ-autoreactivity revealed that either keratinocyte- and intestinal cell-derived proteins or airway epithelium-derived proteins were targeted. This dichotomic pattern was observed in autoreactive sera from atopic patients with AE, food-sensitization, allergic rhinitis and allergic asthma, but never in non-atopic controls.

Conclusions: These findings suggest that auto-IgE is an early marker for the loss of immune-tolerance, which might be helpful for diagnosis of childhood eczema. Moreover, auto-IgE is not organ-specific but targets various proteins. The dichotomic pattern of gut/skin versus airway epithelium autoreactivity in atopic patients suggests that certain, but not all autoallergens play a role in disease pathogenesis. Ongoing analysis of birth cohorts might identify high-risk autoreactivity profiles as a biomarker for subphenotyping of atopic individuals that predicts the atopic march or disease severity for personalized prophylactic and therapeutic approaches.

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A role for CCR3-CCL28 in T cell homing to the upper airway mucosa

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Background: The localization of effector T cells to skin and mucosal tissues is crucial for the generation of effective immune responses, and it has been clearly demonstrated that local production of specific chemokines is important for tissue-specific homing of T cells to the skin and intestinal mucosa. However, molecular mechanisms that regulate the migration of T cells to the human upper airway mucosa are not fully explored.

Aim: Identify which chemokine(s) and their corresponding receptor(s) that is important for homing of $CD4^+$ T cells to the nasal mucosa under homeostatic conditions.

Methods: Tissue samples from normal skin, nasal and intestinal mucosa and blood from healthy individuals were obtained. Gene expression analysis and immunohistochemistry were performed on tissue sections. Immunophenotyping, polyclonal T-cell stimulation and antigen-dependent T-cell proliferation experiments were performed on single-cell preparations of tissue digests and on peripheral blood mononuclear cells.

Results: Among most characterized chemokines (n=39), only CCL28 was preferentially expressed at a high level in the normal nasal mucosa compared with intestinal mucosa and skin. CCL28 was localized to the luminal face of the vascular endothelium. Importantly, 50% of $CD4^+$ T cells residing in the nasal mucosa expressed the CCL28-receptor CCR3, but not CCR10. Conversely, CCR3 was only expressed on a minor population of $CD4^+$ T cells in peripheral blood, small intestine and skin (< 1%). Polyclonal stimulation of single-cell suspensions from the nasal mucosa showed that most of the resident CD4+ T cells produced IFNγ (40%), whereas 10-20% produced IL-17, and 5-10% were $IFN\gamma^-IL\text{-}17^-IL\text{-}2^-$ $Foxp3^+$ T regulatory cells. Only very few CD4+ T cells produced Th2 cytokines. Depletion of $CCR3^+CD4^+$ T cells abrogated the proliferative response of blood $CD4^+$ T cells against the opportunistic nasopharyngeal pathogen *H. Influenzae*, but had no effect on the T-cell response to the systemic antigen tetanus toxoid.

Conclusion: Our results indicate that CCL28-CCR3 interactions are involved in the trafficking of $CD4^+$ T cells to the upper airways under homeostatic conditions. These findings may have important immunotherapeutic implications to regulate the function of effector $CD4^+$ T cells in the upper airways.

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Evidence for functional cross-talk between TLR2 and the high affinity receptor for IgE (FceRI) on human Langerhans cells

Herrmann N, Koch S, Peng WM, Leib N, Wilms H, Schnautz S, Novak N, Bieber T

Background: Atopic dermatitis (AD) is a severe chronic skin disease, with increasing incidence. Patients suffering from early onset AD have a higher risk of developing asthma and allergic rhinitis. We and others have previously shown that in contrast to epidermal Langerhans cells (LC) from healthy donors, LC from AD patients express a trimeric form ($\alpha\gamma_2$) of the high affinity receptor for IgE, FcεRI. It is well known that the skin of AD patients is highly colonized by *Staphylococcus aureus* (SA). Thus we hypothesized that innate receptors for SA may be involved in the pathology of AD by impacting on the functional behaviour of epidermal LC and could show that TLR2 is upregulated on LC in a subgroup of AD patients.

Methods: To study the impact of TLR2 on FcεRI, we established a model of immature LC generated from $CD34^+$ hematopoietic stem cells expressing both receptors.

Results: While there was similar surface-expression of TLR2 on CD34LC from different donors, FcεRI was expressed heterogeneously. We could show that treatment of CD34LC with the TLR2 ligand Pam3Cys induced LC maturation. No early internalization of either TLR2 or FcεRI was observed upon TLR2 ligation and TLR2 surface expression was little affected after 24 h. Surface FceRI was downregulated rather on mRNA level, as we could pinpoint by quantitative PCR. Additionally we could show that mRNA of both components of the trimeric FcεRI receptor (ag_2) was reduced in CD34LC after stimulation with Pam3Cys.

Conclusion: Taken together, we show a direct impact of TLR2 stimulation on the expression profile of FcεRI on LC. This interaction may contribute to the pathophysiological role of LC in AD.

Oral Abstracts Session 4

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Characterization of allergen-specific T cell responses in humanized allergy mice

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Background: In the past, T cell receptor (TCR) transgenic (tg) mice with a murine TCR specific for chicken ovalbumin in the context of a murine restriction element ($I\text{-}A^d$) have been frequently used in allergy research to investigate the contribution of T cells to the priming and/or maintenance phase of allergic diseases. While these models were helpful to some degree, they are not relying on human-relevant respiratory allergens.

Methods: To build a solid foundation for human-relevant allergen-specific T cell studies we have resorted to clone allergen–specific TCRs and we have created a double transgenic mouse model consisting of a human *Artemisia vulgaris* major pollen allergen (Art v 1)-specific TCR (TRAV17/TRBV18) and human MHC molecules (HLA-DRA*01:01/-DRB1*01:01). High expression levels were achieved by chimerizing the allergen-specific human TCR variable sequences with murine TCR constant sequences. Murine TCR alpha and beta promotor/enhancer elements guided the expression of the chimerized transgenes from pT_{cass} vectors. Once established, the allergen-specific TCR tg founder mice were cross-bread to HLA-$DR1^+$ B10.M-$DR1^{dlAb1\text{-}Ea}$ mice.

Results: 89.8±6.9 % of blood $CD4^+$ T lymphocytes of double tg TCR/HLA-DR1 mice showed clear-cut expression of the Art v 1-specific TCR while $CD14^+$ monocytes and $B220^+$ B lymphocytes revealed typical HLA-DR1 expression. Moreover, splenocytes of TCR/HLA-DR1 double tg mice proliferated well upon incubation with the human-relevant immuno-dominant Art v $1_{25\text{-}36}$ peptide or whole Art v 1 protein. In marked contrast, no specific proliferation was evident when splenocytes of control mice were used, or when splenocytes of double tg mice were incubated with irrelevant control peptides or proteins. The effect on T cell phenotype and specific antibody production of *in vivo* challenge with specific allergen or control proteins/peptides will be described and discussed.

Conclusion: Humanized allergy models, in which major constituents of the allergen-specific synapse formed between APC and T cells are defined at the molecular level will contribute to the further understanding of the pathophysiology of human allergic diseases.

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Effects of a new potent poly(ADP-ribose)polymerase inhibitor in guinea-pig allergic asthma

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Background: Poly(ADP-ribose)polimerases (PARPs) are a family of abundant enzymes that catalyzes the covalent attachment of poly(ADP-ribose) (PAR) from NAD to target proteins. PARP-1 is the most abundant of these proteins, it is located in cell nuclei and is able to modulate the expression of a number of inflammatory genes. The asthmatic airway inflammation is regulated by an elaborated network of inflammatory cytokines. Our previous studies demonstrated that PARP is involved in allergen-induced bronchoconstriction. Here we have studied the effects of hydroxyl-dimethylaminomethyl-thieno[2,3-c]isoquinolin-5(4H)-one (HYDAMTIQ) in an in vivo model of asthma-like reaction in the guinea pig.

Methods: Ovalbumin (OA)-sensitized guinea pigs, treated i.p. for 7 days with HYDAMTIQ at the dose of 1,3 and 10 mg/kg b. wt or

with saline, were placed in a respiratory chamber and challenged with OA (5mg/ml in saline) for 60 sec. Respiratory parameters were recorded and quantified. After 48 h the animals were anesthetized and ventilated with a small-animal respirator and challenged with MeCh (0.1 mg/ml in saline) for 60 sec. Changes in the pressure at the airway opening (PAO) were registered. At the end, bronchial airway liquid (BAL) was collected for PAR expression, prostanoid and cytokine level determinations. Lungs were removed histology and for myeloperoxidase (MPO), caspase-3 activities, 8-OH-d-guanosine (8-OH-d-G), malonyldialdeyde (MDA) and MnSOD determinations as well as lung eosinophil infiltration.

Results: Cough and dyspnea in response to OA aerosol were absent in naïve animals whereas they became severe in sensitized guinea pigs. In the latter ones, OA aerosol induced bronchial constriction and pulmonary air space inflation. Treatment with HYDAMTIQ dose-dependently reduced cough, dyspnea, bronchoconstriction and PAO. In cells obtained from BAL and in lung tissue, we found increased PAR expression, mast cell degranulation, MPO activity, MDA and 8-OH-d-G production. We also found a significant increase on PgE_2, PgD_2, LTB_4 and TNFα and IL_{10} in BAL fluid. On the contrary, MnSOD was decreased. Treatment with HYDAMTIQ reduced PAR expression and restored the levels of MnSOD activity. It also decreased all the other above mentioned parameters.

Conclusions: The present findings suggest that PARP inhibitors could be a promising approach to alleviate asthma attack and may provide background for future clinical trials to evaluate the possible anti-asthmatic potential of PARP inhibitors.

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Bitter taste receptors (TAS2Rs) are expressed on leukocytes from asthmatics and increased in children with severe asthma

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Background: Bitter taste receptor (TAS2R) agonists cause bronchodilation in human and mice airways (Deshpande et al Nature 2010). We investigated the expression of TAS2Rs in leukocytes from children and adults with asthma and the mode of action of TAS2R agonists in airway smooth muscle.

Methods: An unbiased Genome Wide Transcriptome Analysis (Affymetrix Human Gene ST 1.0 chip) was first performed in peripheral white blood cells from children with controlled asthma (CA, n=20) and severe, therapy-resistant asthma (SA, n=20) and compared to the results in age-matched healthy controls (Ctrl, n=19). Next, RT-PCR for 11 TAS2Rs was examined in isolated lymphocyte, monocyte and neutrophil fractions from 12 well-characterised adult asthmatic subjects. The profile of actions and the signaling at TAS2Rs was investigated in the isolated guinea-pig trachea (GPT).

Results: In the children with asthma, 1378 genes were differentially expressed in one or several of the CA vs. Ctrl, SA vs. CA or SA vs. Ctrl contrasts. Among significantly enriched KEGG pathways, *TAS2Rs* were most prominently up-regulated in SA, and an unsupervised hierarchical clustering of the differentially expressed genes separated the SA, CA and Ctrl individuals with TAS2Rs being the strongest driving factor.

Expression of several TAS2Rs was replicated in mixed leukocytes from the adult asthmatics and analysis of the fractions showed that expression of all receptors was greatest in the lymphocyte fraction, with hTAS2R10 being most abundant.

The TAS2R agonists denatonium, thiamine, noscapine and chloroquine induced concentrationdependent relaxations of GPT pre-contracted with carbachol (CCh), and expression of their corresponding receptors were identified by RT-PCR in GPT. The magnitude of denatonium-induced relaxation (57.5±5.2%) was enhanced by indomethacin (97.7±2.3%) and the prostaglandin E2 (PGE2) receptor (EP1) antagonist ONO-8310 (99.3±0.7%). In contrast, chloroquine, was unaffected by indomethacin. The effects of denatonium, but not those of chloroquine were partly inhibited by iberiotoxin, charybdotoxin or paxilline, blockers of the large Ca2+ activated K+ channels (BKCa). Precontraction with supramaximal concentrations of CCh was relaxed by denatonium but not by albuterol.

Conclusions: The increased expression of bitter taste receptors in severe asthma suggests a protective role for the receptors that also may be a new target for therapy.

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Active soluble ADAM33 in bronchoalveolar lavage fluid is associated with airway remodelling and bronchial hyperresponsiveness in allergic airway inflammation mouse models

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Background: *A Disintegrin and Metalloprotease-33 (ADAM33/Adam33)* is an asthma susceptibility gene associated with bronchial hyperresponsiveness (BHR). In bronchoalveolar lavage fluid (BALF) from asthmatic patients, soluble forms of ADAM33 (sADAM33) containing the metalloprotease (MP) are increased and inversely correlated to FEV_1%, suggesting a role in airway remodelling in asthma. Exogenous IL-13 or maternal allergic airway inflammation suppress *ADAM33/Adam33* mRNA expression in human embryonic and new-born mouse lungs but enhance ADAM33 protein processing in the mouse lungs. Therefore, we hypothesised that IL-13 or house dust mite (HDM) induced allergic airway inflammation induces the release of active sADAM33 in BALF and this is associated with BHR.

Methods: Mixed background mice were sensitised and intra-tracheally challenged with HDM. IL-13 induced lung inflammation was induced by feeding Doxycycline to double transgenic (dTG) and single transgenic (sTG) CCSP-rtTA/Otet-IL-13 mice (6-8 weeks; males) for 7 days. Assessment of BHR in response to Methacholine was performed. Lungs were harvested for RT-qPCR and immunohistochemistry (IHC). BALF was obtained for Western-

blotting for ADAM33 and its enzymatic activity was tested by a fluorescence resonance energy transfer (FRET) peptide assay.

Results: In response to Methacholine, BHR was significantly increased in HDM challenged and dTG IL-13 mice compared to controls. Airway inflammation and staining for airway smooth muscle was increased in dTG lung sections. *Adam33* mRNA expression was significantly suppressed in dTG IL-13 mice with no difference in α-*smooth muscle actin* (α*Sma*) mRNA, similar to previous findings. However, Western blotting in BALF showed a 76kDa protein consistent with the ectodomain of ADAM33 and 2 smaller forms (38/44kDa) in HDM challenged and dTG mice, suggesting the presence of processed forms. FRET assay with ADAM33 specific peptide demonstrated a significant increase in enzymatic activity in BALF from dTG mice.

Conclusion: HDM and IL-13 induced lung inflammation suppressed *Adam33* mRNA but induced the release of sADAM33, and increased enzymatic activity in the airways. Release of sADAM33 may play a role in airway remodelling by increasing bronchial smooth muscle leading to increased BHR. The effect of specific ADAM33-MP inhibitors merits further study in murine and human models of asthma and may identify potential novel treatment approaches.

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From metabolome to function: Low molecular weight factors from pollen are modulators and aggravators of the allergic immune response in vitro and in vivo

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Background and aim of the study: Evidence is compelling that non-allergenic, adjuvant substances from pollen drive the immune system towards a pro-allergic immune response. Previous studies showed that pollen derived lipids modulate dendritic cell (DC) functions in a way leading to the establishment of a Th2-dominated immune response against pollen. As these effects could not solely be attributed to the previously identified pollen-associated lipid mediators, the pollen metabolome was analysed for candidate immune-modulatory substances. Furthermore, the *in vivo* relevance of these non-allergenic substances in acute allergic inflammation was delineated in clinical tests.

Methods: Protein free fractions of aqueous pollen extracts (APE) were generated by ultrafiltration and were subjected to metabolome analysis (ultra-high resolution mass spectrometry) and ultra-performance liquid chromatography. A low molecular weight fraction of APE (APE<3kDa) was used to study effects of pollen-associated adenosine on monocyte-derived DC´s cyclic AMP signalling, cytokine response and capacity to differentiate T helper cells. Furthermore, in *in vivo* studies, birch pollen-allergic patients were pricked on the forearms with an allergen-containing fraction of APE, supplemented with either PBS or the low molecular weight fraction of APE or adenosine. Wheal and flare size were measured. Additionally, repetitive nasal provocations were performed with birch pollen allergic patients using either allergen-containing or allergen-free extracts. IL-8 was measured in nasal secretions. Primary and secondary clinical end points were total nasal symptom score (TNSS), peak nasal inspiratory flow (PNIF) and cumulative nasal secretion volume.

Results: Ultra-high resolution mass spectrometry revealed that the <3kDa fraction of aqueous pollen extracts comprised thousands of substances, including adenosine in micro molar concentrations. Functional *in vitro* assays showed that pollen-derived adenosine mediated A_2-receptor-dependent induction of cAMP and inhibition of IL12 in human DCs. DCs of non-atopic donors exposed to APE showed an adenosine-dependent reduced capacity to differentiate Th1 cells and an enhanced capacity to induce Treg and IL10. Notably, DCs of atopic donors were less efficient in differentiating functional Tregs in the presence of pollen derived adenosine.

Human *in vivo* studies of the effector phase of the allergic immune response revealed that when prick tests were performed with the allergen fraction of APE supplemented with APE<3kDa, wheal and flare sizes were significantly larger than with the allergen fraction alone. Adenosine was identified as one of the responsible factors of the aggravating effect observed. Concurrently, patients nasally provoked with the allergen-fraction plus APE<3kDa reported a higher TNSS than patients challenged with the allergen-containing fraction only.

Conclusion: Low molecular-weight fractions of APE e.g. pollen derived adenosine exert a dual role in the different phases of the allergic immune response. In the initiation phase pollen derived adenosine exerts a tolerogenic effect – however, only in non-allergic individuals. Due to a still unidentified defect allergic individuals develop a Th2 instead of Treg response. In the elicitation phase, low molecular weight factors from pollen aggravate both cutaneous and nasal allergic responses in response to the allergen. Delineating the exact mechanisms and pathways of immune modulation/aggravation by the adjuvant factors from pollen will help us to develop new therapeutic options in anti-allergic therapy.

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Histamine-releasing factor (HRF) in asthma and atopic dermatitis

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Background: Effects of monomeric IgE on mast cell biology exhibit vast heterogeneity, depending on IgE used. Highly cytokinergic (HC) IgEs can induce many activation events such as degranulation, cytokine production, etc, whereas poorly cytokinergic (PC) IgEs can induce only weak survival promotion (Asai et al., Immunity, 2001; Kitaura et al., PNAS, 2003). Because of the reminiscence to the heterogeneity in the ability of IgE molecules to prime basophils in response to HRF, we examined the relationship between HRF and IgE.

Method: ELISA, GST pulldown and flow cytometry, as well as in vitro mast cell stimulation and in vivo airway inflammation were used.

Results: We found that a subset of IgEs bind to HRF via their Fab portions; the IgE-binding sites were mapped to two different sites of HRF, indicating that dimeric HRF can cross-link IgE-bound FcεRI; N19 and H3 peptides derived from IgE-binding sites blocked HRF-Ig interactions; administration of these inhibitors drastically reduced airway inflammation in asthma models (Kashiwakura et al., JCI, 2012).

Moreover, HC, but no PC, IgEs exhibit polyreactivity to HRF and other autoantigens (Kashiwakura et al., submitted). Interestingly, sera from atopic dermatitis (AD) patients showed increased reactivity to autoantigens and increased levels of HRF. Some AD patients, but not healthy individuals, had high serum levels of HRF-reactive IgE. AD sera with high titers of autoreactive IgE could induce increased IL-8 secretion from human mast cells.

Conclusions: Blockade of HRF-Ig interactions may represent a potential prophylactic and therapeutic strategy for the treatment of asthma. Our results show support the autoimmune mechanism in the pathogenesis of atopic dermatitis.

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Histamine receptor 2 regulates respiratory allergy and inflammation

Liam O'Mahony

Background: Histamine is a biogenic amine with well described effects relating to many of the symptoms associated with allergy and asthma. However, the influence of histamine on various important immunological cell types, such as antigen-presenting cells (APCs), Natural Killer (NK) cells, epithelial cells, T lymphocytes and B lymphocytes is not completely understood. Histamine and its four g-protein coupled receptors (H1R – H4R) represent a complex system of immunoregulation with distinct effects dependent on receptor subtypes and their differential expression. These are influenced by the stage of cell differentiation as well as micro-environmental influences, leading to the selective recruitment of effector cells into tissue sites accompanied by effects on cellular maturation, activation, polarization and effector functions leading to tolerogenic or pro-inflammatory responses. The aim of this study was to investigate the role of H2R in murine models of allergic airway inflammation.

Method: Female H2R-/- mice and BALB/c wild-type mice were sensitized i.p. and OVA-aerosol challenged. The H2R antagonist Famotidine, or the H2R agonist dimaprit, were administered on the same days as OVA sensitization and challenge. Bronchoalveolar lavages (BALs) were obtained for measurement of cellular phenotypes, while lung tissue was digested and single cell suspensions generated. Lung cell phenotypes were analyzed by flow cytometry or stimulated *in vitro* with OVA to measure cytokine secretion.

Results: In mice, blocking H2R using small molecule inhibitors leads to more severe respiratory inflammation in the OVA-model, characterized by enhanced BAL eosinophil numbers, increased numbers of mast cells within lung tissue and elevated secretion of proinflammatory cytokines by *in vitro* stimulated lung cells. In contrast, activating H2R during OVA sensitization and challenge is protective. In addition, H2R-/- mice showed significantly increased cell numbers in BALs, mainly due to elevated eosinophil numbers. Lung histology confirmed increased inflammatory scores in knockout animals. Furthermore, *in vitro* re-stimulation with OVA induced higher Th1 and Th2 cytokine release from lung and spleen single cell suspensions. Lastly, there is altered recruitment and activation of regulatory cells and effector cells within the lung of H2R-/- mice, as measured by flow cytometry.

Conclusions: H2R is an important immunoregulatory receptor that influences the severity of allergic airway inflammation in murine models. Elucidation of the molecular mechanisms underpinning histamine receptor cross-talk with immune-relevant pathways (e.g. TLR signaling) will identify novel therapeutic targets for allergic diseases.

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Role of IL-33 in allergy

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IL-33, a member of the IL-1-related cytokines, is considered to be a pro-allergic cytokine that is especially involved in Th2-type immune responses. Moreover, like IL-1a, IL-33 has been suggested to act as an "alarmin" that amplifies immune responses during tissue injury. In contrast to IL-1, however, the precise roles of IL-33 in immune responses and disease development are poorly understood. Therefore, we investigated the role of IL-33 in allergic diseases using IL-33-deficient mice. The expression of IL-33 mRNA was increased in the local lesion of T cell-mediated type IV hypersensitivity such as contact and delayed-type hypersensitivity. However, IL-33 is not essential for the development of contact and delayed-type hypersensitivity, since these diseases were normally observed in IL-33-deficient mice. On the other hand, IL-33 was important for innate-type mucosal immunity in the lungs. That is, IL-33 was essential for manifestation of T cell-independent protease allergen-induced airway inflammation as well as OVA-induced allergic topical airway inflammation, without affecting acquisition of antigen-specific memory T cells. These observations indicate that IL-33 is a crucial amplifier of innate, rather than acquired, immune responses in lungs.

Oral Abstracts Session 5

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Local polyclonal IgE in nasal polyps with asthma is functional

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Background: Chronic rhinosinusitis with nasal polyps (NP) is often associated with asthma. Both diseases share many features such as airway eosinophilia, a TH2 cytokine profile and local IgE formation. Recent evidence shows that the level of tissue inflammation and local IgE formation is independent of the presence of allergy, but dependent of local IgE to Staphylococcus aureus enterotoxins.

Methods: Local IgE production in NP tissue was shown by qPCR for eGLT and by ImmunoCAP for total and specific IgE. The functionality of local IgE was demonstrated by in-vitro allergen stimulation on nasal polyp explants and by an in-vivo nasal allergen provocation in thirty NP patients with and without allergy compared to patients with allergic rhinitis.

The final proof of the functionality of local IgE in NP was provided by injection of omalizumab in a randomized double-blind, placebo-controlled study in allergic and non-allergic patients (n=24) with nasal polyps and asthma.

Results: In NP, local IgE and key markers of local class switching are elevated compared with normal controls. We demonstrated that nasal tissue IgE is functional and that tissue mast cells can be degranulated after in vitro stimulation of nasal polyp tissue with allergens (such as grasspollen), however the magnitude of the reactivity was less in tissue of allergic NP compared to allergic rhinitis. This finding was confirmed in the nasal provocation study in patients with AR and NP.

In a DBPC trial with omalizumab we demonstrated a significant decrease in total nasal endoscopic polyp score after 16 weeks in the omalizumab-treated group compared to placebo, irrespective of the presence of allergy.

Conclusions: We demonstrated local IgE production in NP with asthma. Both the in vitro and in vivo allergen provocation studies confirmed the functionality of this local IgE in NP with asthma. The reduced reactivity is most likely due to the polyclonality of local IgE in NP.

Finally, anti-IgE treatment had a beneficial effect on NP score, airway symptoms and on the quality of life scores in both allergic and non-allergic patients with nasal polyps and asthma, supporting the importance and functionality of local IgE in the airways.

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Correlation between basophil responsiveness and nasal symptoms following allergen challenge or seasonal exposure in grass allergic patients

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Background: Specific immunotherapy (SIT) trials are currently based on natural allergen exposure. Standardized procedures for nasal allergen challenge as well as a correlation to seasonal symptoms have to be established before nasal allergen challenge can be used to monitor the clinical effect of SIT.

Furthermore, a clear link between nasal symptom scores and cellular immunological responses would make it possible to use these readouts as biomarkers of the clinical effect of SIT.

Methods: 40 grass allergic patients where challenged with *Phleum pratense* allergen extract using the Aptar BiDose system (100 ul/5000 SQ-U to each nostril). Total Nasal Symptom Score (TNSS) 30 minutes post challenge was compared to average symptom scores during season (4 weeks peak season, miniRQLQ).

Basophils in whole blood were stimulated with various concentrations of *Phlp* extract and CD63 and CD203c expression was monitored by flow.

Treg effects were investigated by *Phlp*-specific stimulations of co-cultures of $CD4^+CD25^+/CD127^{lo}/foxp3^+$ Treg and $CD4^+$ Teff or of Treg-depleted $CD4^+$ T-cells.

Correlations were sought by Spearman rank test between challenge and seasonal nasal symptoms, between symptom scores and various readouts for basophil activation (sensitivity, total activity, and maximal activation) or between the different readouts for basophil or T-cell activation/inhibition.

Results: Modest (r values = 0.344-0.475), but significant correlations (p values = 0.03-0.003) were found comparing various individual scores between pre-seasonal challenge and seasonal exposure to grass allergens.

In addition, significant correlations (r = 0.35-0.58, p = 0.0003-0.03) were found between basophil pre-seasonal reactivity and selected seasonal and nasal challenge symptom scores.

Highly significant correlations were found between most basophil readouts but not between sensitivity and maximal activation. Significant inhibition of allergen-specific Teff cell proliferation was observed in Treg-Teff co-cultures while only a trend was found in depletion experiments.

Conclusion: The correlation between nasal symptoms induced by standardized nasal challenges and nasal symptoms in seasonal suggests that controlled challenges may be used to monitor the effect of SIT. The correlation between the level of basophil activation and clinical readouts further suggest that this immunological test may be a relevant biomarker in SIT trials. Finally, quantification of Treg function should be investigated in Treg-Teff co-cultures.

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Susceptibility of atopic dermatitis (AD) model mice to vaccinia and herpes simplex viruses

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Background: AD is a chronic inflammatory skin disease. The etiology of this disease is multifactorial, and involves complex interactions between genetic and environmental factors. The underlying defective skin barrier allows the penetration of allergens and microbial pathogens. AD patients have a propensity to develop severe viral infections such as eczema vaccinatum and eczema herpeticum caused by vaccinia virus (VACV) and HSV, respectively. We established and characterized the pathophysiology of these disease models, using AD-prone NC/Nga mice.

Method: Dermatitis was induced by epicutaneous application of allergen and Staphylococcal enterotoxin B in NC/Nga mice, followed by virus inoculation into the eczematous lesions. Genomics, immunological and virological examinations were performed.

Results: Global gene expression profiles were similar in the skin between our AD model and human AD patients, supporting the clinical relevance of our model. Virus infection of eczematous skin induced severe erosive skin lesions, but not in the skin of healthy mice. Eczematous mice exhibited lower natural killer (NK) cell activity. The role of NK cells in controlling VACV- or HSV1-induced skin lesions was demonstrated by experiments depleting or transferring NK cells. The proinflammatory cytokine IL-17 reduced NK cell activity in mice with preexisting dermatitis. Given low NK cell activities and increased IL-17 expression in AD patients, these results can explain the increased susceptibility of AD patients to virus infection.

Conclusions: We established mouse models of eczema vaccinatum and eczema herpeticum, characterized their immune responses to virus infection, and showed the importance of NK cells in early suppression of VACV- or HSV1-induced severe skin lesions.

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Aggregated IgG activates human synovial mast cells from rheumatoid arthritis or osteoarthritis patients through Fc gamma RI

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Background: Substantial evidence suggests that human synovial mast cells (MCs) are involved in the pathogenesis of rheumatoid arthritis (RA). A plausible pathway for the activation of synovial MCs is through IgG receptors, given the prevalence of circulating IgG isotype autoantibodies and synovial immune complexes in patients with RA. However, IgG receptor expression on human synovial MCs remains uncharacterized. The present study sought to identify which IgG receptor(s) on synovial MCs are responsible for MC activation in immune complexes.

Method: Synovial tissues were obtained from patients with RA or osteoarthritis (OA) undergoing joint replacement surgery, and the synovial MCs were enzymatically dispersed. Synovial cultured MCs were then generated by culturing synovial cells with stem cell factor, and receptor expression was analyzed using flow cytometry. The function of MCs through Fc receptors was monitored by measuring mediators.

Results: Primary synovial MCs and cultured synovial MCs obtained from both RA and OA patients expressed FcεRI, FcγRI, and FcγRII, but not FcγRIII. The aggregation of FcγRI on primary synovial MCs induced degranulation. Cultured synovial MCs induced degranulation and the production of PGD_2 and TNF-α (808.3 ± 301.5 pg/ml/MCs^6) following aggregation of FcγRI using anti-FcγRI mAb (10 μg/ml) plus anti-mouse $F(ab')_2$ fragments of IgG (0.3 μg/ml). The aggregation of FcγRII caused histamine release from cultured MCs, but not from primary MCs. Histamine release induced by aggregated IgG was significantly inhibited by neutralizing anti-FcγRI mAb, but not by anti-FcγRII mAb.

Conclusions: The Fc receptor expression profiles of synovial MCs from RA and OA patients were similar. FcγRI was responsible for producing abundant TNF-α from synovial MCs in response to the aggregation of IgG. Immune complexes may activate synovial MCs through FcγRI.

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Transplacental transmission of respiratory syncytial virus (RSV)

Giovanni Piedimonte

Background: Transplacental transmission of viruses like cytomegalovirus (CMV), rubella, and HIV is well documented in humans, and results frequently in fetal morbidity or mortality. However, prenatal infection with RSV has never been studied and no association has been made with neonatal outcome. We have tested the hypothesis that RSV is transmitted across the placenta from the infected respiratory tract of the mother to the fetus, and that this virus modifies the expression of growth factors critical for the development of lung innervation.

Methods: Midterm pregnant (day 12 of a 21-22 days gestation) Fischer-344 dams were dosed with 1.5 $x10^6$ PFU of red fluorescent protein (RFP)-tagged RSV or sterile medium via intratracheal instillation. Five days post-infection, the dams were sacrificed and the uterus was removed and washed with saline. Fetuses were removed, placed in ice-cold sterile media, and processed for flow cytometry measurement of RFP-tagged RSV infected cells and nerve growth factor (NGF) protein. Flow cytometry measurements were made on red blood cell-free single cell suspensions. Measurements of were acquired with FACSCalibur and analyzed with CellQuest Pro software. Other fetuses were homogenized, sonicated in cell culture media and centrifuged. The supernatant was then passed through sterile 0.2 μm syringe filter, placed on a monolayer of epithelial cells and incubated at 37°C with 5% CO_2 for 3-4 days.

Results: Half of the fetuses from RSV-infected dams were positive for RFP-tagged RSV by flow cytometry analysis, compared to none of the fetuses from control dams. NGF expression was positively correlated with RSV infection in all fetuses tested. Seventy-five percent of the epithelial cell cultures incubated with extracts of fetuses from RSV-infected dams became infected, whereas none of the cultures incubated with extracts of fetuses from pathogen-free dams became infected.

Conclusions: RSV was vertically transmitted from the infected lungs of the dam to the fetus. The infection was detected by flow cytometry and cell culture in 50% and 75% of fetuses borne by infected mothers, respectively. A concomitant increase was detected in NGF protein concentration. Although there was no obvious increase in fetal mortality, sequelae of this intrauterine infection in childhood and adulthood are being assessed in an ongoing study.

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The effects of inhaled interferon β on viral exacerbations in asthma

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Exacerbations of remain a major unmet clinical need especially in those with severe disease. In 1995 we first reported that respiratory viruses especially rhinoviruses were responsible for the majority of asthma exacerbations in children and later confirmed this in adults. We further showed that the airway epithelium was the principle target for all RV subtypes and, using *in vitro* epithelial cultures, that in contrast to normal cells, those from asthmatics enabled viral replication and cell cytotoxicity on account of a reduced capacity to generate interferon (IFN) β when microsomal TLR3 is activated by viral dsRNA, the primary innate antiviral defence. Because the asthmatic molecular "lesion" was shown to be in the first step of the IFN signalling mechanism, leaving the second amplification step intact, it was possible to restore anti-viral protection to asthmatic epithelial cells *in vitro* by addition of small amounts of exogenous IFNβ. Based upon this observation we have pursued the clinical development of inhaled to protect against severe viral exacerbations. After confirming safety, inhaled IFNβ1a in asthma was shown to activate the anti-viral pathways revealed by dose-related elevation of the biomarkers neopterin and IP-10 in sputum and elevation of the genes in sputum cells encoding MxA, IP-10 and 2'5'-OAS. We then proceeded to a placebo-controlled clinical trial in 147 moderate-severe asthmatic (BTS Sign Steps 3-5, Gina Steps 3 & 4) patients. At the onset of a suspected viral infection inhaled IFNβ1a or placebo was administered daily for 14 days and patient related and lung function outcome measures followed during this period and for 5 weeks follow-up. PCR-base viral detection in sputum showed that 68% of patients were infected with RV the other viruses being adenovirus, bocavirus, coronavirus, enterovirus, HMPV, parainfluenza and RSV. In those receiving placebo there was a close correlation between upper severity of upper and lower respiratory tract symptoms. The antiviral biomarker IP-10 in sputum increased in both active and placebo groups over 48 hours, but only in the IFNβ-treated group was this sustained during the 2 week period of active treatment and associated with an ~10-fold reduction in viral load. Efficacy was shown only in those with severe asthma (BTS 4 & 5) with a marked protection against worsening Asthma Control Questionnaire score (primary end point) (p=0.003 on day 8), PEF, symptom score, β_2-agonist use and the percentage of subjects experiencing a moderate exacerbation (p<0.01). There were no adverse events linked to the active treatment. We conclude that Inhaled IFNβ is an effective way of treating viral exacerbations in patients with severe asthma by mechanisms that intervenes on the causal pathway.

Poster Abstracts Session 4

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Evidence for T cell independence of secondary IgE responses in allergic patients

Katharina Marth, Eva Wollmann, Daniela Gallerano, Portia Ndlovu, Ian Makupe, Rudolf Valenta, Elopy Sibanda

Background: The infection of CD4+ cells by HIV leads to the progressive destruction of CD4+ T lymphocytes and, after massive reduction of CD4+ cells, to AIDS.

To study if HIV-infected patients suffering from AIDS with a severe reduction of CD4+ cells can suffer from symptoms of IgE-mediated allergy and produce allergen-specific IgE antibody.

Methods: In total 69 HIV-infected allergic patients from Zimbabwe were studied. Among these patients, 27 had CD4 counts below 200. Allergy was diagnozed according to case history, physical examination and skin prick testing. Serological analysis of allergen-specific IgE antibodies was performed with an allergen chip, containing 163 purified allergen molecules or with the MAST-CLA® assay, containing a panel of 36 allergen extracts. IgE antibody levels specific for seasonal allergens (Art v 1, Art v 3, Bet v 1, Cup a 1, Cyn d1, Ole e1, Phl p 1) were quantified with ImmunoCAP measurements when follow-up sera obtained at different time points were available. HIV infection was confirmed serologically and the disease was staged clinically. Determination of CD4+ and CD8+T lymphocyte subset numbers were performed by flow cytometry.

Results: The predominant allergic symptoms of HIV-infected patients were IgE-mediated symptoms such as allergic rhinoconjunctivitis and urticaria whereas T cell mediated symptoms (e.g., atopic dermatitis) ceased in patients with very low CD4 counts. In accordance to the clinical symptoms IgE responses specific for house dust mite, grass pollen and moulds were most frequent. ImmunoCAP measurements of IgE levels specific for seasonal allergens indicated that even patients with CD4 counts <200 exhibited boosts of IgE production.

Conclusion: Our results indicate that allergen-specific IgE production and immediate IgE-mediated allergic inflammation do not require a fully functional CD4+ T lymphocyte repertoire.

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Micro-array based analysis of allergic and non-allergic individuals over ten years suggests absence of spontaneous de novo IgE sensitizations in adults

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Background: The frequent observation that adult allergic individuals gain clinical reactivity to new allergen sources over time has never been elucidated before with respect to changes in IgE reactivity profiles to purified allergen molecules. Using allergen extracts it is not possible to distinguish between genuine *de novo* sensitizations and the boosting and spreading of pre-existing IgE-reactivities. In the present study we used an allergen micro-array to investigate whether there are spontaneous changes of IgE reactivity profiles in sensitized adults and in non-allergic individuals over a period of 10 years upon natural allergen exposure.

Methods: Sera of 12 allergic and 10 non-allergic individuals were collected in 1997 and 2007. Allergen-specific IgE was detected using an allergen-microarray containing 85 purified allergen molecules. Presumptive *de novo* IgE sensitizations, i.e., IgE-reactivities against allergen families that were detected in 2007 but not in 1997, were reassessed by highly sensitive ImmunoCAP measurements.

Results: The determination of IgE reactivities to 85 different allergen molecules representing 50 families of cross-reactive allergens in 12 allergic and 10 non-allergic individuals over a period of ten years showed that there was no *de novo* sensitization to new allergen families. In one patient the possible new sensitization to one single allergen molecule (Act d 2) could not be re-investigated by quantitative IgE measurements since the respective ImmunoCAP was not available.

Conclusions: Our results indicate that in adults under conditions of natural allergen exposure *de novo* sensitizations to new allergens are unlikely events. This finding has important implications for the prescription and development of antigen-specific and patient-tailored treatment strategies.

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Development of an allergic rhinitis model induced by Japanese cedar pollen in BALB/c mice as an experimental model

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Background: Japanese cedar (Cryptomeria japonica) pollinosis is one of the most serious type I allergic diseases caused by Japanese cedar pollen (JCP) in spring. In recent decades, it is prevalent in 10 to 30% of the Japanese population and poses a significant social problem. In patients suffering from rhinitis, symptom as sneezing and nasal blockage has been reported. To date, there have been a few reports on murine allergic rhinitis models which failed to elicit increased the number of sneezing. Therefore, this study aimed to try and develop an allergic rhinitis, sneezing, in the mouse.

Method: JCP-extract was prepared. To characterize the dried JCP-extract, major allergens (Cry j 1 and Cry j 2) were detected by SDS-Page, and their concentrations were measured by ELISA. For *in vivo* study, female Balb/c mice (7 weeks old) were used. Mice were sensitized by i.p. administration of JCP-extract with alum three times at intervals of one week. To confirm the sensitization, antigen-specific IgE antibody was measured in mouse serum by ELISA. Three weeks after the final sensitization, mice were given multiple intranasal (i.n.) instillations of JCP-extract with either histamine or PBS pretreatment. After 5 exposures to i.n. JCP challenge, the frequency of sneezing was observed by visual inspection.

Results: The protein content was determined and corresponded to 0.16 mg protein/mg JCP-extract. Following SDS-PAGE, the proteins migrating at 40 and 45 kDa were identified as Cry j 1 and Cry j 2. ELISA showed major allergen concentrations of 6.9 and 2.7 μg/mg protein for Cry j 1 and Cry j 2, respectively. JCP-specific IgE levels in serum were higher following JCP-extract/alum treatment than JCP-extract/saline treatment. Significantly, increases in the number of sneezes were only observed in JCP-extract/histamine-pretreated mice, not in JCP-extract/PBS mice.

Conclusion: Sensitization was successfully induced by JCP-extract/Alum. JCP-extract challenge was not sufficient to increase sneezing frequency in sensitized-mice. However, it was improved following histamine pretreatment. Although further study is required, it is possible that histamine is likely to prime the mucosa with regard to subsequent allergic reactions. The immunological parameters are under the investigation.

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Mast cell dependent and independet airway hyperresponsiveness

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Background: Allergic asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), inflammation and tissue remodelling. Clincial studies and results from animal models suggest that mast cells play a central role in the pathology of asthma. In the present study, we tested if mast cells influence the AHR in a chronic murine model of asthma.

Methods: C57BL/6 (wild-type) and mast cell-deficient B6.Cg-*Kit*$^{W\text{-}sh}$ mice without (Wsh) and with (Wsh+MC) mast cell-engraftment were sensitized to and subsequently challenged with ovalbumin for a 91 days period. AHR was measured using the flexiVent system and the forced oscillation technique which enables the separation of central and peripheral airways.

Results: In wild-type mice, pulmonary mast cells were localized in the submucosa of the central airways, whereas the more abundant mast cells in Wsh+MC mice were found mainly in

the alveolar parenchyma. In Wsh+MC, OVA challenge caused a relocation of mast cells from the perivascular space and central airways to the parenchyma. Allergen challenge caused a similar AHR in wild-type and Wsh mice, which were located in the resistance of the airways and the pulmonary tissue. This AHR was pronounced in Wsh+MC mice, which also demonstrated enhanced tissue elastance. The elevated functional responses were related to the numbers and localization of connective tissue-type mast cells in the peripheral pulmonary compartments. A mast cell-dependent increase in IgE and IL-33 together with impairment of the IL-23/IL-17 axis was evoked in Wsh and Wsh+MC mice by allergen challenge.

Conclusion: This study show that within the same chronic murine asthma model the development of AHR is both dependent and independent of mast cells. Moreover, the spatial distribution and number of pulmonary mast cells determine severity and localisation of the AHR.

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Prostaglandin D2 augments IgE-dependent histamine release from mast cells through E prostaglandin receptor, EP3

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Background and purpose: Mast cells play a central effector role in the development of allergic inflammatory reactions by producing and releasing a wide variety of preformed mediators, de novo synthesized lipids, and many cytokines and chemokines in response to IgE-dependent stimulation. Of these various inflammatory mediators they produce, prostaglandin D_2 (PGD_2) is known as a major prostanoid produced by the activation of mast cells. Recently, some PGs are reported to be important endogenous regulators of mast cells activity; however, the effects of PGD_2 on IgE-mediated activation of mast cells have not been fully elucidated.

Experimental approach: In the present study, we investigated how PGD_2 modulates IgE-dependent mast cell activation via autocrine and/or paracrine pathway using bone marrow-derived mast cells (BMMCs) of mice deficient in the DP, CRTH2, FP or EP3 receptors and each receptor selective agonist.

Key results: In wild-type BMMCs, PGD_2 significantly and dose-dependently enhanced IgE-dependent histamine release from BMMCs. Although each mRNA of these receptors was expressed in BMMCs, the enhancement of IgE-mediated degranulation by PGD_2 was dependent on the EP3 receptor. Similarly, ONO-AE-248, an EP3 selective agonist, significantly and dose-dependently enhanced IgE-mediated release of histamine by BMMCs of wild-type animals, and the enhanced response to IgE-Ag by ONO-AE-248 treatment was completely abolished in EP3-deficient BMMCs. Furthermore, pertussis toxin, a Gi-protein inhibitor, completely inhibited the enhancement of IgE-dependent histamine release from mast cells.

Conclusion and Implication: These results clearly demonstrated that the enhancement of IgE-mediated degranulation of mast cells by PGD_2 is mediated by Gi-coupled EP3 receptor as a pro-inflammatory signaling pathway via autocrine and/or paracrine fashion.

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Role of histamine H_4R in bleomycin-induced pulmonary fibrosis

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Background: Pulmonary fibrosis is a progressive and lethal illness characterized by inflammation and abnormal remodeling of lung parenchyma. No cure exists for this disease. Various are the models used for the study of pulmonary fibrosis, among these, the bleomycin one is the best characterized murine model in use. Bleomycin alters oxidant/antioxidant balance and ROS overproduction activates several intracellular signaling pathways, leading to pro-inflammatory cytokine production. The histamine H_4R, expressed on cell of immune origin, plays an important role in inflammatory process. We previously demonstrated that JNJ7777120 (JNJ), a selective H_4R antagonist, potentiates the beneficial effect of naproxen in this experimental model. The aim of the present study was to investigate the role of different H_4R ligands in controlling inflammation and pulmonary fibrotic process induced by bleomycin.

Methods: C57/bl6 mice were treated with vehicle, JNJ (total dose 40 mg per Kg/bw) or ST-1124 (partial inverse agonist), ST-1006 (partial agonist),ST-994 (neutral antagonist) and ST-1012 (inverse agonist) at equimolar doses, released by micro-osmotic pumps for 21 days. Airway resistance to inflation, an index of lung stiffness, was assayed and lung tissue processed to evaluate oxidative stress (malonyldialdeady (TBARS), 8-OH-d-guanosine (8-OH-d-G), and inflammation (myeloperoxidase (MPO),COX_2 expression, PgE_2) markers as well as fibrosis (tissue growth factor-β (TGF-β), % of positive goblet cells, thickness of smooth muscle layer)

Results: Our results indicate that JNJ, ST-994 and ST-1012 exert an anti-inflammatory effect, as shown by the significant decrease of the levels of PGE_2, MPO, an index of leukocyte infiltration, and TBARS, markers of oxidative stress. They also reduce the relative number of goblet cells, the thickness of smooth muscle layer (parameters of inflammation-induced adverse bronchial remodeling), the level of pro-fibrotic cytokine TGF-β and collagen deposition; these effects are accompanied by a decrease in airway resistance to inflation.

Conclusion: Our results indicated that H_4R blockade is associated with an anti-inflammatory and anti-fibrotic effect and may offer a new therapeutic option for the treatment of Th2-dependent lung inflammatory disease.

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Alteration of intestinal microbiota increases mortality to respiratory viral infection in an IFN-gamma dependent mechanism

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Recent studies have indicated that alterations in composition of the gastrointestinal microbiota can affect the immune system in the intestinal tract and other mucosal sites. Using our model of paramyxoviral induced atopic disease, we examined whether gastrointestinal microbiota alteration affected the immune response and subsequent atopic disease. To alter intestinal microbiota, mice were given water with or without the non-absorbable antibiotic streptomycin (0.5g/250mL water). Mice were intranasally inoculated with a non-lethal dose of Sendai virus (SeV, 2 x10^5 pfu). Addition of streptomycin to drinking water for ≥ 2 weeks before viral inoculation led to a marked increase in mortality (81% versus 0%, n≥23; p<0.0001). High-throughput pyrosequencing demonstrated marked reduction in intestinal bacterial diversity with streptomycin treatment. Using qPCR it appeared that the *Firmicutes* phylum was most affected. Streptomycin treated mice that survived to day 21-post inoculation (p.i.) SeV were noted to have increased mucous cell metaplasia (p<0.02). The presence of inflammatory cytokines was quantified by ELISA based cytokine array at 8 days p.i. Increased levels of IFNγ (1343+/-439.9 versus 305.8+/-118.1 pg/mL, mean+/-SEM, n≥10; p=0.015), IL-6 (4891+/-483 versus 2945+/-431.5 pg/mL, n≥5; p= 0.015) and CCL3 (1504+/-212.9 versus 985.1+/-158.2 pg/mL, n≥10; p= 0.048) were found in the BAL fluid of streptomycin treated versus untreated mice. These increased pro-inflammatory cytokines were associated with decreased regulatory T cells (Treg). Treg numbers in lung were decreased by 47% with streptomycin treatment (1.05+/-0.13 x10^5 versus 2.0+/-0.18 x10^5; n≥3; p=0.01), and Treg cells in the distal small intestine were decreased by 92% with streptomycin (0.157+/-1.28 x10^6 versus 1.97+/-0.44 x10^6 with no streptomycin; n=3; p=0.03). To determine if IFNγ caused the increased mortality, we administered an IFNγ blocking antibody (200 µg SQ) on days 5 and 9 p.i. SeV. Blocking IFNγ completely prevented streptomycin increased mortality (0% versus 60% mortality, anti-IFNγ mAb versus control IgG, n=5 per group; p<0.05). Alteration of the intestinal microbiota before SeV infection led to increased mortality due to increased IFNγ production in the presence of markedly reduced Tregs. These data suggest Tregs may link gastrointestinal and respiratory mucosal immune systems, especially in response to respiratory viral infections.

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Serum 25-hydroxyvitamin D level and atopy in Korean

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Introduction: Lower vitamin D levels were associated with increased airway hyper-responsiveness (AHR) and higher eosinophil counts and IgE levels, while higher vitamin D levels were associated with a lower hospitalization for asthma exacerbations.

Objective: This study was conducted to evaluate whether atopy had relation with serum vitamin D level in Korean population.

Methods: We analyzed data obtained as part of the fifth South Korean National Health and Nutrition Examination Survey (Korean NHANES V, 2010) of 8,958 Koreans. The correlation between serum 25-hydroxyvitamin D level and presence of atopy with results of specific IgE to Dermatophagoides farina (Df), cockroach and dog hair using Phamacia CAP system (Phamacia, Uppsala, Sweden) was analyzed. All estimates were calculated on the basis of the sampling weight.

Results: Mean age was 37.80 years and male were 50.2%. Subjects with serum specific IgE to Df, cockroach or dog hair were 48.2%. Mean serum 25-hydroxyvitamin D level was 17.66±0.27 ng/mL. There was no difference in serum 25-hydroxyvitamin D level between populations with atopy and without atopy (17.97±0.35 vs. 17.97±0.32 ng/ml, P>0.05). Despite of no difference in serum 25-hydroxyvitamin D level according to presence of atopy to Df or dog hair, population with specific IgE to cockroach had higher vitamin D level compared to those without specific IgE to cockroach (18.76±0.44 vs. 17.53±0.30 ng/ml, P=0.005). Total IgE level was positive correlation with serum 25-hydroxyvitamin D level (r=0.145, P<0.001)

Conclusion: In the current study, atopy status was not related with serum vitamin D level in Korean.

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In preschool children with acute wheeze, human rhinovirus group C infection is the most common cause and the only virus associated with atopy

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Background: Recent studies have shown that human rhinovirus (HRV) group C (HRV-C) is the most common cause of acute wheezing exacerbations in young children and suggest it is more pathogenic than HRV-A or B. Given the emerging importance of HRV-C in early childhood wheezing, we examined the relationship between HRV-C and atopy.

Aim: To determine the relationship between virus isolated and atopy in preschool children presenting to an emergency department (ED) with acute wheezing.

Hypothesis: In young children with acute wheezing, HRV-C is more closely associated with atopy than other viral groups.

Methods: 214 children aged 0-5 years presenting to a tertiary children's hospital's ED with an acute wheezing illness were prospectively recruited as part of the MAVRIC (Mechanisms of Acute Viral Respiratory Infections in Childhood) study. Two separate control groups were recruited: 76 healthy community children; and 49 siblings of the acute case recruited contemporaneously.Skin reactivity to common allergens was performed and a nasal aspirate was collected at recruitment. Viral RNA was extracted, reverse transcribed and tested for all common respiratory viruses. In addition, a two-step PCR of the HRV 5'NCR confirmed HRV detection, followedby sequencing for typing.

Results: As previously noted, HRV-C was substantially more common in cases (48.5%) than healthy controls (8.7%)(p <0.001), whereas HRV-A and HRV-B were no more frequent in cases than controls (HRV-A 19.8%, 17.4%; HRV-B 1.8%, 7.2%, respectively). However, in the cases, atopy was associated with HRV-C (p<0.009), but not any other virus (including HRV-A, HRV-B,respiratory syncytial virus (RSV) (table, * % of atopic cases).

	Acute cases	Community controls	p
n	214	76	
Age (months) (SD)	32.5 (16.7)	34.0 (14.1)	0.44
Atopic (n)	84	16	
HRV-A *	17.6	33.3	0.26
HRV-B *	0	33.3	0.01
HRV-C *	60.3	11.1	<0.009
RSV *	5.9	0	1.00

The separate comparison between acute cases and sibling controls produced similar results. Results remained significant after adjustment for confounders.

Conclusions: HRV-C was the most common virus causing acute wheezing exacerbations in young children and the only viral group associated with atopy. These findings have strong implications regarding the relationship between atopy and asthma.

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Effect of the selective COX—2 inhibitor etoricoxib on allergen—induced airway obstruction and profile of lipid mediator metabolomics in bronchial provocation of subjects with asthma

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Background: Prostaglandins (PG) that constrict and relax airways are biosynthesised in reactions catalysed either by cyclooxygenase isoenzymes 1 or 2 (COX-1 and COX-2). In mice models of asthma, COX-2 inhibition aggravates airway hyperresponsiveness (AHR). It is unclear if inhibition of COX-2 in subjects with asthma selectively removes PGs that make asthmatic responses better or worse.

Methods: Fifteen subjects with mild atopic asthma , AHR to methacholine (MCh) and demonstrated allergen-induced bronchoconstriction, were challenged with rising doses of allergen and MCh on two different days to determine the provocative dose causing 20% drop in FEV1 (PD20) during a control session and following 10-12 days of treatment with the COX-2 inhibitor etoricoxib (90 mg once daily). Induced sputum was collected before and after the allergen challenges. The effect of the treatment was validated biochemically by measurements of thromboxane generation in clotted blood for COX-1 and LPS-induced formation of PGE2 in leukocytes for COX-2, respectively. Urine was collected before and after allergen provocations for establishment of treatment effects on the excretion of 30 key metabolites of PGs, leukotrienes and isoprostanes by mass spectrometry.

Results: Etoricoxib treatment did not change pre-challenge FEV1 (mean ± SEM FEV1 3.78 ± 0.20 and 3.82 ± 0.20 after drug and control, respectively), nor AHR to MCh or allergen (PD20 values). Neither was the maximum fall in FEV1 different after treatment with etoricoxib (mean ± SEM % drop in FEV130.4±1.3 and 25.3± 3.6 after drug and control, respectively), nor the allergen induced increase in sputum eosinophils post challenge. The biochemical assays confirmed the effectiveness and COX-2 selectivity of the treatment and discovered that allergen-challenge increased formation of several isoprostanes.

Conclusion: This first study of a COX-2 inhibitor in the allergen-challenge setting found no negative effects of etoricoxib on airflow obstruction and sputum eosinophils induced by the challenge, basal lung function or MCh responsiveness in subjects with atopic asthma. Short-term use of COX-2 inhibitors may thus be safe in asthmatics. Mechanistically, the data support that bronchoprotective PGE2 is derived from COX-1 and also that there is significant oxidative stress during the response to allergen.

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Dose response relationship between ascaris sensitisation with aeroallergen sensitisation and airway hyperresponsiveness but not allergic diseases in an urbanising teenage African population

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Background: We sought to examine the relationship between sensitisation to helminths and atopy, airway hyperresponsiveness and allergic diseases in an African cohort where both worms and atopy abound.

Methods: Xhosa children living in a peri-urban environment were tested for ascaris IgE levels, airway hyperresponsiveness (AHR) by methacholine challenge, atopic sensitisation (skin tests to aeroallergens) and allergic disease (asthma, eczema and rhinitis) assessed by questionnaire.

Results: Ascaris sensitisation was strongly associated with AHR but not with asthma, eczema or rhinitis. There was a dose-response relationship between increasing class of ascaris IgE and increased AHR (Prevalence ratio (PR) 1.75; CI 1.09-2.82). Higher levels of ascaris IgE were seen in those with AHR.

Ascaris IgE was associated with atopic sensitisation to aeroallergens. There was a significant dose-response relationship between increasing class of ascaris IgE and sensitisation to one or more allergen (PR 1.65), sensitisation to house dust mites (HDM) (PR 1.79) and grass (PR 2.66). The strong association between ascaris class and any positive SPT remained even after sensitisation to HMD and grass was excluded (PR 1.61). The number of positive SPT's increased by 78% for every level of ascaris IgE class increase from 0 to 2.

Subjects with sensitisation to ascaris had a higher number of positive SPTs (mean 0.92; median 1; IQR 0-2) than those with no ascaris sensitisation (mean 0.42; median 0; IQR 0-0). In addition ascaris sensitisation was associated with more than doubling the prevalence of HDM sensitisation (41.5% vs 18.5%) and almost quadrupling the prevalence of grass sensitisation (10.8% vs 2.8%).

Ascaris IgE levels were higher in those with HDM sensitisation compared to those without.

Conclusions: Ascaris sensitisation was strongly associated with aeroallergen sensitisation and airway hyperresponsiveness but not asthma, eczema or rhinitis. These associations were dependent on the level of ascaris-specific IgE in a dose-dependent fashion. Possible explanations might be that the type of ascaris infection that causes high levels of ascaris IgE in this genetic population may also favour the development of atopy or that atopics in Africa have upregulation of their defence system against parasitic infection. These hypotheses are not mutually exclusive.

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Different phenotypes of exercise induced bronchoconstriction and associated factors: 8-year retrospective analysis of free running provocation test

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Background: Exercise induced bronchoconstriction (EIB) is one of the characteristic features of bronchial asthma and limits the daily activity of asthmatic patients. EIB is recently classified into EIB only and EIB with asthma, however, the difference of these two entities is now clearly investigated yet.

Methods: A retrospective chart review was conducted on patients with a history of asthma and respiratory symptoms during exercise. Six-minute free running challenge test was performed and more than 15% decrease of forced expiratory volume in 1s (FEV1) from the baseline value was interpreted as positive exercise bronchoconstriction. The temperature, humidity, and sensitization to inhalant allergens were compared according to the presence of airway hyperresponsiveness (AHR) to methacholine.

Results: Exercise induced bronchoconstriction was observed in 89 patients (66.9%). EIB positive subjects showed higher positivity to methacholine provocation test (61.4% vs. 18.9%, p<0.001), outdoor mold in skin prick test (24.1% vs. 6.5%, p=0.040) compared with EIB negative subjects. Sputum eosinophilia was also more frequently observed in EIB positive subject than EIB negative subject (56% vs. 23.5%, p=0.037). The temperature and humidity of the exercise test day were significantly related with positive rate of EIB. However, these associations persisted in EIB only group but not in EIB with AHR to methacholine. Positive EIB was correlated with temperature (p=0.001) and relative humidity (p=0.038) in the methacholine negative group while such a correlation was not observed in the methacholine positive group (p=0.844 for temperature, p=0.787 for humidity). The time to reach 15% fall of FEV1 was significantly earlier in the methacholine positive group compared with the methacholine negative group (3.2±0.7 vs. 8.6±1.6 minutes, p=0.004) and maximal fall of FEV1 was also grater in the methacholine positive group than the methacholine negative group (32.62±1.7 vs. 24.63±2.1, p=0.005).

Conclusions: EIB only is a distinct clinical entity from EIB with asthma. Conditions such as temperature and humidity should be considered on performing the of exercise test of EIB only.

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Monitoring asthma with a smart-phone application

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Background: Asthma patients may experience acute episodic exacerbation. The guidelines recommend that written action plan should be given to asthma patients. However, no one can predict when and where acute exacerbation will happen. As people carry smart phone almost anytime and anywhere, smart phone application may be useful tool in asthma care. We evaluated the usefulness of the ubiquitous healthcare system of asthma care system using smart-phone application (snuCare®).

Methods: Forty-four patients including fragile asthmatics were enrolled from Seoul National University Bundang Hospital between December 2011 and February 2012. They were randomly assigned into application user (22) or application non-user group (22). We evaluated user-satisfaction, and clinical parameters such as Asthma control test (ACT), Quality of Life Questionnaire for Adult Korean Asthmatics (QLQAKA), and the adherence of patients.

Results: The characteristics were similar at baseline between two groups except BMI (user vs. non-user 22.8 vs. 25.8, p=0.003) and those who treated with short-term systemic steroid or increased dose of systemic steroid during previous 8 weeks (user vs. non-user 31.8% vs. 4.5%, p=0.020). Total of 2,226 signals was generated during 8 weeks including five risky states. After eight weeks, the users answered that it was very easy to use the application, which was shown in highest scores in terms of satisfaction (4.3±0.56). Seventy three percent of patients answered that the application

was very useful for asthma care. User group showed improved the adherence scores (p=0.017). Those who treated with short-term systemic steroid or increased dose of systemic steroid during 8 weeks were similar (user vs. non-user 22.7% vs. 13.6%, p=0.440). One patient in application user group could avoid emergency department visit owing to the application while a patient in non-user group visited emergency department.

Conclusion: The ubiquitous healthcare system using a smart-phone application (snuCare®) could be helpful in the monitoring and the management of asthma.

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A novel technique for house dust mite inhalation in an Allergen Challenge Chamber – a validation study in patients with allergic rhinitis

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Background: There is an increasing demand for studies on indoor allergens, particularly on house dust mite (HDM) allergens, in order to perform basic clinical research and to test anti-allergic treatments under well-controlled conditions. A novel technique to universally create an atmosphere loaded with allergen particles was developed. In brief, aerosols of defined size distribution are produced by spray-drying of an aqueous solution containing a licenced allergen extract and lactose as a carrier. The particle size distribution can be adjusted and depends on the initial droplet size spectrum and the lactose concentration in the solution. For a pilot study in patient with allergic rhinitis particles with size above 10 µm are generated for preferential nasal challenge.

Objective: The aim of this study was to test the safety and efficacy with dose-range finding and reproducibility of this novel HDM challenge in patients with allergic rhinitis

Methods: In a monocenter, placebo-controlled, single-blind, five-way cross-over pilot study 18 subjects with allergic rhinitis and sensitization to HDM were exposed in the Fraunhofer Challenge Chamber for 4 hours to house dust mite allergen (ALK lyophilised SQ503 Der p, ALK-Abello, Wedel, Germany) at either 250 SQE/m^3, 500 SQE/m^3, 1000 SQE/m^3, or lactose alone (0 SQE/m^3) seven days apart. The dose of 500 SQE/m^3 was repeated to investigate reproducibility. Total nasal symptom score (TNSS), anterior rhinomanometry, nasal secretions, exhaled NO, FEV_1 and adverse events were assessed prior to and during the exposures.

Results: At a lactose concentration of 10% (w/v) the mass median aerodynamic diameter of allergen particles was 13.5 µm with only 11% of aerosol mass in the size range below 10µm. Exposure to HDM increased TNSS (mean±SD) to 3.4±1.8, 3.3±2.1, and 3.6±2.0 at 250 SQE/m^3, 500 SQE/m^3, and 1000 SQE/m^3, respectively, while lactose alone did not change TNSS (0.7±0.6). Repeated exposure to 500 SQE/m^3 induced a TNSS of 3.0±2.2 which was not different compared to the same previous dose. Objective measures of nasal flow and nasal secretions were in line with clinical symptoms. Exposure to HDM was safe with no relevant change in FEV_1.

Conclusion: HDM allergen challenge in the Fraunhofer Challenge Chamber using a novel technique to universally generate allergen atmospheres was safe and specifically induced symptoms in patients with allergic rhinitis due to HDM. Symptoms were reproducible but no dose-dependency was observed in the tested dose-range. This pilot study provides the methodological basis for further clinical studies using well controlled allergen atmospheres.

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Peripheral T cell signature after nasal allergen challenge in allergic rhinitis

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Rational: Several studies have demonstrated the time course of inflammatory mediators in nasal fluids following nasal allergen challenge (NAC), whereas the effects of NAC on T cell signature in peripheral blood are unknown. We hypothesized that NAC is associated with priming and activation of plasmacytoid dendritic cells (pDCs), and that the frequency of grass pollen-specific Th2 T cells is increased, independent of changes due to diurnal variation.

Methods: We conducted an observational study outside the grass pollen season. 12 patients with grass pollen-induced seasonal allergic rhinitis underwent diluent/saline challenge followed by NAC 14 ± 7 days later. Total nasal symptoms scores (TNSS) and peak nasal inspiratory flow were recorded. Plasmacytoid dendritic cells (pDCs) and T lymphocytes were assessed in peripheral blood using flow cytometry and fluoroSpot assays before and at 3, 6 and 24 hr after NAC and on a control day following diluent challenge.

Results: Mean TNSS were significantly increased (AUC, control day: 12.9 ± 4.7; NAC day: 212.0 ± 20.7). CD303+CD123+CD80+ (p=0.08) and CD303+CD123+CD86+ (p=0.02) pDCs were elevated in peripheral blood at 6 hours. A significant increase in CRTH2+CD4+ T cells (p=0.003), CD4+CD25lo (p=0.003) and CD4+CD152+ (p=0.008) T effector cells was observed at 6 hour post NAC but not following control challenge. Grass pollen-driven CD4+ T cell proliferative responses and frequency of Th2 cells by Flourospot assay (IL-4+ CD4+ T cells) were significantly increased at 6hr after NAC when compared to control day (p= 0.01 and p=0.02).

Conclusions: Nasal allergen challenge *in vivo* is associated with increases *in vitro* in grass pollen-driven CD4+ T cell proliferative responses and increased frequency of allergen–driven Th2 (IL-4+CD4+ T cells) in peripheral blood. These potential biomarkers could be useful in monitoring treatment responses in allergic rhinitis.

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The efficacy and safety of a short course of budesonide inhalation suspension via transnasal nebulization in chronic rhinosinusitis with nasal polyps: a randomized, placebo-controlled study with immunologic evaluation

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Background: There is little scientific evidence to support the use of topical glucocorticosteroid via transnasal nebulization to treat patients with chronic rhinosinusitis with nasal polyps (CRSwNP). The objective of current study is to evaluate the efficacy and safety of a short course of nebulized budesonide via transnasal inhalation in patients with CRSwNP.

Methods: In a placebo-controlled trial, we randomly assigned sixty patients with CRSwNP to receive either nebulised budesonide suspension (1 mg twice daily, transnasal inhalation) or placebo for 14 days. Visual analogue scales (VAS) of nasal symptoms (including nasal obstruction, nasal discharge, loss of smell, and headache/facial pain) and endoscopic polyp scores and morning serum cortisol concentration were performed to both groups before and after the treatment. Nasal polyp biopsies were obtained at baseline and after 14 days. The level of cytokines such as IL-5, IL-8, IL-10, IL-17, Interferon (IFN)-γ, Eotaxin, Transforming growth factor (TGF)-β were measured in polyp tissue homogenates. Single-cell suspensions of mononuclear cells were isolated immediately from nasal polyp after biopsy, and the frequencies of different T cell subsets in polyp tissue were evaluated by flow cytometry.

Results: Nebulized budesonide inhalation caused a significant improvement in all nasal symptoms especially nasal obstruction ($p < 0.001$) and reduced polyp size compared with placebo. The morning serum cortisol concentration was no different after 14 days treatment in nebulized steroid group compared with baseline, and all values were located in normal range (normal range: 5 - 25μg/dl). Nebulized budesonide via transnasal inhalation significantly reduced the levels of IL-5 and Eotaxin in nasal polyp tissue homogenates, whereas it increased levels of IL-10 and TGF-β. The frequency of Th2 cells ($CD4^+IL\text{-}4^+$ T cells) in $CD4^+$ T cells in nasal polyps was reduced significantly, but that of Tr1 cells ($CD4^+IL\text{-}10^+IL\text{-}4^-$ T cells) was increased after treatment. The frequency of natural T regulatory cells ($CD4^+CD25^+Foxp3^+$ T cells) and Th1 cells ($CD4^+IFN\text{-}\gamma^+$ T cells) was no different before and after treatment in the steroid treatment group.

Conclusion: A short course of nebulized budesonide transnasal inhalation improved rapidly nasal symptoms, polyp size, and did not cause HPA axis suppression; it displayed a significant immunological effect via up regulating the production of IL-10 and TGF-β and the frequency of Tr1 cells in local mucosa.

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Alleviation of sneezing and nasal rubbing in a murine model of allergic rhinitis by intranasal administration of Semaphorin 3A

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Abstract: Sneezing and persistent itching of the nasal mucosa in allergic rhinitis (AR) are distressing symptoms. Recent studies have revealed that hyperinnervation of sensory neurons in the nasal turbinate is one of the underlying causes of sneezing and itching. It is presumed that reduced Semaphorin 3A (Sema3A) expression in the nasal mucosa might contribute to the hypersensitivity, becausee Sema3A has been previously shown to restrict innervation of sensory neurons. Analysis of the mouse model of ovalbumin-sensitized AR demonstrated a decreased expression of Sema3A in the nasal epithelium, which was accompanied by an increased nerve fiber density in the lamina propria of the turbinate. In this mouse model of AR, intranasal administration of recombinant Sema3A alleviated sneezing and nasal rubbing symptoms. In addition, histological examinations also revealed that nerve fiber density was decreased in the lamina propria of the Sema3A-treated nasal turbinate. These results indicate that the nasal hypersensitivity of AR may be attributed to reduction of Sema3A expression and intranasal administration of Sema3A may provide a novel approach to the treatment of these allergic symptoms in AR.

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Aberrant expression of Semaphorin 3A and nerve growth factor in the epidermis is associated with itch in the skin lesions of not only atopic dermatitis but also prurigo nodularis and psoriasis

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Atopic dermatitis (AD) is a representative pruritic chronic inflammatory skin disease that is triggered by an interaction between the genetic constitution of the individual and stimulation by allergens and irritants. Prurigo nodularis (PN) is a pururitic chronic or recurrent skin disease characterized by the severe scratching and multiple hyperkeratotic papulonodules. Psoriasis vulgaris (PV) is also another pruritic chronic inflammatory skin disease. Pathophysiology of itch has been studied during the past 10 years, but the mechanism is not still fully elucidated in each pruritic disease. Recently it has been shown that C-fibers are ectopically innervated in the epidermis associated with the increased expression of nerve growth factor (NGF) and decreased expression of Semaphorin 3A (Sema3A) in skin lesions of AD compared with healthy skin. Furthermore, we investigated the effect of Sema3A on the skin lesions and their itch by injecting the recombinant Sema3A intralesionally in NC/Nga mice, an animal model of AD, and revealed that Sema3A dose-dependently attenuated the scratching behavior and improved the skin lesions.

However, such aberrant expression of NGF and Sema3A in these pururitic chronic skin diseases such as PN and PV has not been fully studied. In this study, the expression levels of these 2 molecules in biopsy specimens from the skin lesions of PN and PV and healthy skin were quantified by immunohistochemistry and quantitative reverse-transcription PCR. Sema3A expression was lower in the samples from PN and PV compared with the healthy samples, whereas NGF was higher. C-fibre innervation in the epidermis was also increased in theskin lesions from PN and PV. Sema3A mRNA expression was negatively correlated with itch intensity and severity of PN and PV. We propose that the decreased Sema3A and increased NGF expression levels may trigger the outgrowth of C-fibres, leading to pruritus in these pururitic chronic skin diseases as well AD.

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Improvement of dryness and pruritus in the aceton-induced dry skin model by oral administration of collagen tripeptide

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Dry skin in patients with atopic dermatitis (AD) is supposed as an important factor inducing development and exacerbation of AD, because it causes pruritus and excoriation with high frequency. Topical application of an emollient to the dry skin of AD patients is effective to prevent the induction of pururitus with scratching to some extent. However, more effective and simpler therapies for the dry skin of AD are expected. Collagen tripeptide (CTP) is a highly purified, non-antigenic, low-allergenic collagen fraction that is known to have various biological effects such as an increase of water content in the skin. Therefore, we examined the therapeutic effects of CTP for dry skin using acetone-induced dry skin model mice.

CTP enhanced hyaluronic acid production in human dermal fibroblasts in vitro and in mice skin in vivo. Oral administration of CTP to this dry skin model mice significantly decreased TEWL and suppressed scratching behavior. Intraepidermal nerve growth was dramatically inhibited in CTP-treated mice. Quantitative PCR analysis and immunohistochemical study revealed that the increased NGF and decreased Sema3A levels induced by acetone treatment recovered the almost normal levels of these values in CTP-treated mice. Therefore, it is presumed that oral administration of CTP improves dry skin and normalizes axon-guidance factors in the epidermis in addition to reducing pruritus. CTP may be used in a new therapeutic strategy against the dry skin with pruritus.

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Atopic dermatitis in SubSaharan Africa: Clinical and sensitization patterns in Amaxhosa-speaking patients in Cape Town, SA

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Background: Little is known about atopic dermatitis (AD) in Subsaharan Africa, but recent data show an increasing prevalence in these regions, mainly in urbanized area. However, precise data on clinical features, sensitization patterns and genetic background in this geographic area are still very sparse. Thus we wanted to evaluate the sensitization patterns in a well defined cohort of AD patients and controls.

Methods: 102 subjects of Xhosa ethnic background with AD according to the modified Hanifin Rajka criteria were recruited (Age range 0-49 years, mean age 7 years, f/m 50/52), as well as 105 healthy controls from the same population (mean age 29 years, f/m53/52).. All were clinically examined. Total and specific

IgE levels (ImmunoCAP) for common food and inhalant allergens were measured and a microarray-based assay to define specific IgE against 103 molecular allergens (ImmunoCAP ISAC) was performed. Serology against 7 common parasites and genetic analyses of barrier genes were determined.

Results: AD severity according to EASI was mild in 24 (23.53%), moderate in 44(43.1%) and severe in 34 pts. (33.33%). Total IgE was raised in 91.89% of AD pts. (mean 2216 kU?l) and significantly higher than in controls (elevated in 43.8%, mean 80 kU/l). 28.38% of the AD patients had levels >5000 kU/l, of which 61.9% had severe AD. 89.04% of the patients had a positive specific IgE to at least one allergen, significantly more than in controls (32%, $p < 0.01$). Most common sensitizations in AD pts.were against house dust and storage mites and grass pollen. IgE against food were mostly against egg white (ovalbumin) and - possibly parasite- or raw fish food originating -tropomyosin. There was a strong correlation between AD severity and IgE-mediated sensitizations. 73.7 % of AD patients showed positive parasite serology compared to only 43% in controls, mostly towards Toxocara canis. Barrier genes showed substantial differences compared to Western population s in both groups.

Conclusions: IgE levels and sensitizations in these African Xhosa population are significantly higher in individuals with AD as compared to controls and correlate with clinical severity. Thus also in such geographically and socioeconomic different settings IgE-mediated sensitizations may play an important role in AD, although allergen spectrum and genetic background can differ substantially. The higher parasite infestation may be a contributing factor for the increasing prevalence of AD in urbanized areas.

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Allergic and immunodeficiency findings in atopic dermatitis and hyper-IgE syndrome patients

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Background: Eczematous skin lesions and elevated serum IgE levels are hallmarks of both, severe atopic dermatitis (AD) and hyper-IgE syndromes (HIES). The differential diagnosis between AD and HIES is challenging but important for the patient management and prognosis. Dermatologists and allergists need to be trained to raise suspicion of HIES and to initiate further diagnostic such as molecular testing for HIES. Autosomal dominant HIES is based on heterozygous mutations of the gene *STAT3* (STAT3-HIES) which is more common than autosomal recessive HIES caused by mutations of the genes *DOCK8* and *TYK2*.

Method: A complete personal and family history is advised including questions regarding recurrent infection, pneumonia, and associated findings of HIES. Drawing a pedigree will elute consanguinity and additional affected family members. Key findings for STAT3-HIES are: internal abscesses, pneumatoceles, severe infection, candidiasis, pathologic fractures, scoliosis and positive family history. Symptomatic allergies, especially severe food allergies and recurrent viral infections with HSV and MCV are associated with DOCK8-HIES.

Results: Basic diagnostic procedures should include a differential blood count, immunoglobulin levels of IgA, IgM, IgG and IgE, tests for viral, bacterial and fungal colonization or infection. Depending on the clinical presentation skin prick test, hapten patch test, atopy patch test, in vitro IgE tests, food challenge procedures; lymphocyte phenotyping and function tests, B cell subsets, specific antibody production and molecular diagnostics should be performed. TH17 cells are elevated in AD and reduced in STAT3 patients, while lymphopenia and a low serum IgM level direct to DOCK8-HIES.

Conclusion: Whereas treatment of AD is the key competence of dermatologists and allergists, confirmed HIES should be treated in a specialized center for immunodeficiency diseases. Early therapy is essential for the patients' quality of life and includes emollient therapy, an infection prophylaxis regimen, anti-inflammatory treatment, or even bone marrow transplantation.

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Hymenoptera venom allergy: ICAM-I is overexpressed and decreases after venom immunotherapy

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Background: Adhesion molecules, including ICAM-1, are an important factor in allergic inflammation caused by inhalant allergens, but there are no studies investigating their possible role in Hymenoptera venom allergy.

Method: We measured the level of ICAM-1 in 13 venom-allergic patients before and after ultra- rush venom immunotherapy (VIT). Eight patients were treated by yellow jacket venom and 5 were treated by honeybee venom. Serum ICAM-1 levels were assayed by an immunoenzymatic method, with a detection limit of 0.35 ng/ml.

Results: Mean level of ICAM-1 changed from 316.4±78.2 ng/ml before VIT to 294.7 ± 77.9 after VIT. This difference was statistically significant ($p = 0.019$).

Conclusions: These findings show that in patients with HVA there is an overexpression of ICAM-1, and that ultrarush VIT significantly decreases ICAM-1 levels. It is likely that the known ability of VIT to correct the imbalance in T lymphocytes subpopulations and in the associated production of cytokines may account for this observation. In fact, such cytokines include IL-4 and TNF-alpha, that upregulate adhesion molecules.

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Association between regulation of thymic stromal lymphopoietin receptor expression on peripheral blood dendritic cells and LPS-induced inflammasome activation in healthy children

Yuzaburo Inoue, Taiji Nakano, Naoki Shimojo, Yoichi Kohno

Backgrounds: Thymic stromal lymphopoietin (TSLP) is an interleukin 7-like cytokine expressed mainly by epithelial cells, and has a capacity to activate myeloid dendritic cells (mDCs) to promote Th2-type immune responses. On the other hands, a recent study showed that TSLP can activate plasmacytoid DCs (pDCs) and induce FOXP3$^+$ regulatory T cells in the thymus[1]. Regulation of TSLP receptor (TSLPR) expression on DCs, especially in inflammatory conditions including allergy, is still unclear.

Objective: To delineate the linkage between TSLPR expression and LPS-induced caspase-1 activation in human peripheral blood mDCs or pDCs.

Methods: We collected bloods from 33 children aged 12 years, who did not have asthma or atopic dermatitis. Informed consent was obtained from their parents or guardians. Their peripheral blood mononuclear cells were treated with or without LPS for 6 hours. Cells were stained with fluorescence-labeled caspase-1 inhibitor to detect activated caspase-1. After the treatment, we analyzed expression of TSLPR or high affinity IgE receptor (FceRI) on mDCs (CD14$^-$CD16$^-$ILT3$^+$CD33$^+$CD123$^-$) or pDCs (CD14$^-$CD16$^-$ILT3$^+$CD33$^-$CD123$^+$) by flowcytometry.

Results: Both in mDCs and pDCs, the total serum IgE levels were significantly correlated to FceRI expression, while there was no relationship between the total serum IgE levels and caspase-1 activation, suggesting that susceptibility to LPS-induced caspase-1 activation is independent of atopic status.

In mDCs, LPS-induced changes of TSLPR expression were inversely correlated to increase of activated caspase-1 in the cells. On the other hands, in pDCs, LPS-induced increase of TSLPR expression was positively correlated to LPS-induced increase of activated caspase-1.

Conclusion: Our data suggest that the involvement of susceptibility of LPS-induced caspase-1 activation in the regulation of TSLPR expression may differ between in mDCs and in pDCs. High susceptibility of LPS-induced caspase-1 activation might be related to inhibition of Th2 induction via decreasing expression of TSLPR in mDCs, while it might be related to induction of Tregs via increasing expression of TSLPR in pDCs

[1]Hanabuchi, et al. Thymic stromal lymphopoietin-activated plasmacytoid dendritic cells induce the generation of FOXP3+ regulatory T cells in human thymus. *J immunol.* 2010; 184(6): 2999-3007

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Granulocyte/macrophage colony-stimulating factor-dependent CD11b+ lung dendritic cells are required for induction of T helper 2 immunity to inhaled dust mite allergen

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Although it is well established that dendritic cells (DCs) are essential for allergic immune responses by several mouse models of asthma but the specific role of different lung DC subsets in initiating Th2 immunity remains poorly understood. In this study, C57BL/6 mice were intra-nasally sensitized with *Blomia tropicalis* extract (Blo t) and subsequently challenged via the same route. Bronchoalveolar lavage (BAL) eosinophils and neutrophils were determined by expression of Singlec-F and Ly6-G respectively. Mucus secretion was detected with periodic acid Schiff stain (PAS). T cell cytokines were determined by culture of draining lymph node cells with 20μg/ml Blo t extract for 5 days and assay of culture supernatants by ELISA. This model induced a robust allergic response characterized by high eosinophil (60%) and low neutrophil (10%) infiltration. Hematoxylin and eosin staining identified substantial mononuclear cell infiltration of the lungs and PAS staining demonstrated mucus hyper-secretion and goblet cell hyperplasia. Draining lymph node cells cultured with Blo t extract secreted large amounts of Il-4 (200 pg/ml), IL-5 (2000 pg/ml), IL-13 (4000 pg/ml) and very little IFN-γ (<100 pg/ml). Blo t extract was able to enhance the uptake (10-20% by day 3) and presentation of ovalbumin (OVA) by CD11b$^+$ lung DCs, leading to OVA-specific T cell proliferation and Th2 differentiation in lung draining lymph nodes. When CD11b$^+$ lung DCs were depleted by crossing CD11c-Cre and IRF4-flox mice, the Th2 immune response to inhaled Blo t allergens was attenuated and allergic airway inflammation decreased. Administration of anti-granulocyte/macrophage colony-stimulating factor (GM-CSF)-neutralizing Abs during the sensitization stage markedly reduced Blo t-elicited allergic responses and was associated with reduced antigen presentation capacity of CD11b$^+$ lung DCs. Taken together; these data suggest that GM-CSF-dependent CD11b$^+$ DCs play a critical role in the initiation of Th2 responses to Blo t allergens.

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Plasmacytoid dendritic cells and innate interferons constrain Th2 cytokine responses to rhinovirus: a regulatory mechanism with relevance to asthma

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Background: Respiratory viruses are common triggers of asthma exacerbations. Increasing evidence points to an aberrant anti-viral immune response in asthma, though the mechanisms involved are not well understood. This study aimed to examine the extent to which plasmacytoid dendritic cells (pDC) and interferon (IFN)-α/β regulate immune responses to the human rhinovirus (HRV), and compare type I IFN signalling pathways in healthy and asthmatic donors.

Methods: Blood mononuclear cells were isolated from 18 asthmatic and 18 healthy individuals and cultured with HRV strain 16 for up to 5 days.

Results: HRV induced expression of IFNα, IFNβ and multiple interferon stimulated genes within the first 24h. At 5 days, HRV-specific memory T-cells produced both T_H1 and T_H2 cytokines. Depletion of pDC from HRV-stimulated cultures markedly inhibited IFNα secretion, and led to a significant increase in expression and production of the T_H2 cytokines IL-5 (p=0.02), IL-9 (p<0.01) and IL-13 (p<0.01), but had no effect on IFN-γ synthesis. Neutralising type-I IFN with a decoy receptor led to a similar increase in T_H2 cytokines, but had no effect on IFNγ synthesis. Compared to HRV-stimulated cells from healthy donors, cells from asthmatics exhibited significantly lower expression of IFNβ (p<0.0001), interferon regulatory factors IRF1 (p<0.001) and IRF7 (p=0.03) and reduced secretion of IFNα protein (p=0.005). Numbers of blood pDC and their expression of ICAM-1 were similar in asthmatic and healthy donors.

Conclusions: pDC and the IFNα/β they secrete selectively constrain $T_H2$cytokine synthesis following HRV exposure in vitro. This important regulatory mechanism appears to be dysfunctional in asthma, with multiple deficiencies in innate interferon signalling pathways identified. This has the potential to facilitate allergic airway inflammation and contribute to asthma exacerbations during RV infections.

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CD1a-/CD207-/CD11c+ human dendritic cells bind Bet v 1 allergen within the oral mucosal tissue

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Background: Sublingual allergen-specific immunotherapy has been shown to be a safe and effective treatment for allergic rhinitis. Most likely, antigen-presenting cells (APCs) such as dendritic cells (DCs) play a pivotal role. While in human oral mucosal tissue (OMT) CD1a+ Langerhans cells (oLCs) represent the major DC population involved in allergen uptake, in mice CD11b+ and CD11c+ DCs predominate. However, little is known about CD11c+/CD11b+ DCs in human OMT. Thus, this study focused on DC subpopulations in human OMT and their role in allergen uptake.

Material and Methods: Single cell suspensions from human OMT were obtained by trypsinization. DCs subsets were analyzed by HLA-DR as well as CD1a, CD11b and CD11c expression and investigated for other DCs markers by flow cytometry (FACS). Furthermore, OMT was incubated with FITC-coupled Bet v 1 solution (200 µg/ml in PBS) for 1 hour at 4°C and 37°C prior to culture of OMT in RPMI medium. After 48h, DCs which migrated out of OMT were investigated for Bet v 1 binding by FACS.

Results: We could demonstrate three different DCs subsets within the HLA-DR+ cell population, namely (i) CD1a+/CD207+/CD11c- DCs (15.2% +/- standard error of mean (SEM) 1.9), (ii) CD1a+/CD207+/CD11c+ DCs (6.4% +/- SEM 0.7) and (iii) CD1a-/CD207-/CD11c+ DCs (22.3% +/- SEM 2.4). All DC subpopulations expressed CD1c and CD11b but no other macrophage markers like CD163 or CD68. Moreover CD1a-/CD207-/CD11c+ DCs did not express DCs maturation marker CD83. However, the proportion of CD1a-/CD207-/CD11c+ DCs among HLA-DR+ cells varied between investigated OMT samples (1.9% to 62.3%, n=37). Preliminary experiments (n=3) could show that stimulation of OMT with FITC-coupled Bet v 1 solution led to binding of Bet v 1 mostly to CD1a+/CD207+/CD11c- DCs (95.4% +/- 1.8) and CD1a+/CD207+/CD11c+ DCs (81.8% +/- 9.6) but also to a lower proportion to CD1a-/CD207-/CD11c+ DCs (30.8% +/- SD 9.6).

Conclusion: CD1a-/CD207-/CD11c+ DCs represent a separate DCs subpopulation in human OMT as they are present in variable numbers and did not express oLC associated markers. Although CD1a+/CD207+ oLC appear to be the predominant Bet v 1 binding subpopulation, CD1a-/CD207-/CD11c+ also bind Bet v 1 during absorption ex vivo.

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A new tool for studying intracellular interaction of the dermal mast cells

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Background: Human mast cells which are well established as key players in allergic disease have been recognised lately to be also critically involved in many other physiological and pathological events in skin and other organs. For the study of mast cells, mouse models are frequently used. However a human in vitro model mimicking the complex interaction of dermal mast cells with other major cutaneous cells is lacking. The aim of this study was therefore to establish a serum free long-term co-culture model which includes dermal mast cells, dermal fibroblasts and keratinocytes in a human skin equivalent organotypic setting.

Methods: Primary human dermal mast cells and fibroblasts (1:1) were embedded into a collagen-matrigel gel. After contraction of the gels, primary human keratinocytes was seeded on top of the gels After 24 hours of culture, gels were airlifted to develop a skin like morphology. After one week, keratinocytes had formed an epidermis-like multilayer and were able to proliferate and differentiate.

Results: Immunohistological staining of the 3D-constructs showed strong bromodesoxyuridine and Ki67 positiv basal cells. Expression of the differentiations markers transglutaminase, involucrin and keratin indicated the development of a well differentiated epithelial layer. Expression of ß4-integrin or fibronectin indicated the generation of a basal membrane like structure after two weeks of cultivation. Under these conditions, mast cell integrity and functionality was preserved even after 2 weeks of culture, as shown by immunohistochemistry using antibodies against the c-kit receptor and the mast cell-specific enzymes tryptase, chymase. A decrease of tryptase in response to activation of the mast cells indicated the functionality of these cells.

Conclusions: The absence of tunnel stained cells in the gels indicated low apoptosis rates of mast cells and cutaneous cells in our novel co-culture system. Thus the model presented here provides a novel tool for studying multicellular interaction of dermal mast cells with major other skin cells.

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Expression of a prostaglandin D2 receptor, CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) on human mast cells and potential relevance in allergic diseases

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Background: Prostaglandin D_2 (PGD_2) has long been implicated in allergic diseases such as asthma by contributing to bronchoconstriction, vasodilation, and vascular permeability. Recently, cloning of a second novel PGD_2 receptor CRTh2 (chemoattractant receptor-homologous molecule expressed on th2 cells, also known as DP2), led to a greater understanding of the physiological and pathophysiological implications of PGD_2. PGD_2 signaling through DP1 and CRTh2 (DP2) mediates different and often opposite effects in many cell types of the immune system. Although mast cells (MC) are the largest source of PGD_2 in the body, there is lack of information about their expression of PGD_2 receptors and the functional significance.

Methods: CRTh2 transcripts and protein expression in two human mast cell lines, HMC-1 and LAD2, and two primary cultured human MC, cord blood-derived MC (CBMC) and peripheral blood-derived MC (PBMC), were examined using RT-PCR and flow cytometry. Expression of CRTh2 in MC from human nasal polyps was examined using immunohistochemistry. Intracellular calcium mobilization after treatment with the CRTh2 specific agonist, 15R-15-methyl PGD_2 was measured using Fluo-4NW calcium assay kit. MC degranulation was measured from β-hexosaminidase released into the supernatant.

Results: RT-PCR and flow cytometry showed that human MC express CRTh2. About 35% of tissue MC in nasal polyps expressed CRTh2. The CRTh2 specific agonist induced a dose dependent intracellular calcium mobilization and reduced degranulation in human MC.

Conclusion: Human MC express functional CRTh2. Regulation of MC mediator release through CRTh2-mediated signaling may play an important role in allergic diseases.

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Mast cells control allergic skin inflammation in a chronic type IV hypersensitivity model in mice

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Mast cells (MCs) have been shown to modulate murine skin inflammatory responses to contact allergens. However, the role of MCs in chronic recurrent skin inflammation, the clinical picture in patients with allergic contact dermatitis, has not been investigated in detail. Here, we have studied the role of MCs in skin inflammatory responses to repeated exposure to the contact allergen oxazolone using genetically MC-deficient C57BL/6-*Kit*$^{W\text{-}sh}$/*Kit*$^{W\text{-}sh}$ (Sash) mice. Sash mice showed increasingly enhanced skin inflammation upon repeated challenge with oxazolone, as assessed by measuring ear thickness ($p<0.01$ for the third consecutive challenge). The adaptive transfer of MCs to challenge sites, i.e. the ears, of Sash mice completely repaired this phenotype. Interestingly, enhanced inflammatory skin responses to contact allergen exposure resulted in markedly increased CD4/CD8 double positive T cell populations in the draining lymph nodes of Sash mice as compared to wild type mice. These data point to a crucial role of MCs in the prevention and/or down regulation of type IV allergic skin inflammation induced by repeated allergen challenge, possibly by effects on antigen-specific T cell populations.

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Angiopoietins and their tie receptors in human basophils and mast cells

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Background: Angiogenesis, the formation of new blood vessels from preexisting ones, plays a prominent role in tissue remodeling in several chronic inflammatory disorders. Angiopoietins (Angs) are a novel class of angiogenic growth factors. Ang1, mainly expressed in peri-endothelial cells, promotes angiogenesis binding the Tie2 receptor on endothelial cells. In contrast, Ang2 reduces vascular integrity by competing for the Tie2 receptor. The Tie1 receptor is still considered an orphan receptor. The angiopoietin/Tie system is now recognized to play a substantial role also in the inflammatory process. We sought to characterize Ang1, Ang2, Tie1 and Tie2 expression and functions in human basophils and mast cells.

Methods: Ang1/Ang2 and Tie1/Tie2 expression was evaluated by RT-PCR and flow cytometry in primary human lung mast cells (HLMC), in the mast cell line LAD-2 and in basophils. Ang1 was localized in basophil vesicles by immunogold labelling. Ang1/Ang2 release was evaluated by ELISA. Modified Boyden chambers were used for chemotaxis assay.

Results: Basophils, LAD-2 cells and HLMC constitutively express Ang1 and Ang2 mRNA. Intracellular staining for Ang1 and Ang2 was stronger in basophils than in mast cells. Immunoelectron microscopy demonstrated Ang1 in cytoplasmic vesicles of basophils. The protein kinase C activators phorbol diester (PMA) and bryostatin 1 (Bryo1) stimulated basophils to rapidly release a large amount of Ang1. PMA-induced Ang1 release correlates with histamine and β-hexosaminidase secretion and was inhibited by brefeldin A. Tie1 and Tie2 mRNAs were expressed in basophils, LAD-2 and HLMC. Basophils, LAD-2 and HLMC expressed Tie1 on the cell surface. HLMC and LAD-2 expressed Tie2 on the cell surface, whereas basophils did not. Ang1, but not Ang2, induced

migration of mast cells through the engagement of Tie2. Neither Ang1 nor Ang2 induced basophil chemotaxis.

Conclusions: We have identified a novel mechanism of cross-talk between human basophils and mast cells mediated by the Ang1/Tie2 system that might be relevant in the orchestration of inflammatory angiogenesis in allergic diseases.

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Eicosapentaenoic acid and decosahexaenoic acid suppress Th2 cytokine expression through distinct pathways in RBL-2H3 basophilic cells

Mirim Jin

Background: It has been known that the intake of omega-3 fatty acids, including eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA), has beneficial effects for the prevention and treatment of allergies; however, the cellular and molecular mechanisms have been incompletely understood. Basophils generate a large amount of cytokines with pro-Th2 functions such as IL-4 and IL-13 under various IgE-dependent or independent stimuli, which are critically involved in the onset and exacerbation of allergic inflammatory responses.

Method: In PMA and ionomycin treated RBL-2H3 basophilic cells the inhibition of IL-4 and IL-13 expression by EPA and DHA were examined using ELISA and real-time PCR. The gene promoter activities were measured using luciferase assay in the cells transiently transfected with IL-4 and IL-13 proximal promoter reporter plasmids. Western blot analysis was performed to investigate the involvement of several transcription factors required for IL-4 and IL-13 expression.

Result: EPA and DHA inhibited the IL-4 and IL-13 production of PI-activated RBL-2H3 cells in a dose dependent manner, and that the mRNA levels of IL-4 and IL-13 were significantly decreased. EPA and DHA almost completely suppressed IL-4 and IL-13 gene promoter activities. EPA selectively suppressed NF-AT1 expression in the nucleus, while DHA affected c-Fos, indicating that they might facilitate different pathways. Both EPA and DHA rarely affected the nuclear expression of NF-AT2, NF-κB p65, c-Jun and c/EBPα. Additionally, nuclear expression of GATA-1 and GATA-2 as well as their mRNA expressions were not affected.

Conclusion: Taken together, our data suggested that EPA and DHA might have suppressive effects on allergic responses by the inhibition of IL-4 and IL-13 expression through distinct pathways in basophils.

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Induction of Th2 cytokines by active Def f 1 in mouse bone marrow-derived basophils

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Background: Der f 1, a major allergen of house dust mite, belongs to the papain-like cysteine protease family whose proteolytic activity can provoke TH2 immune responses. Our purpose is establishing the mechanism of TH2-mediated allergic reactions. Herein, we primarily examined IL-4 and IL-13 production by the stimulation of proteolytically active Der f 1 in the mouse bone marrow-derived basophils (BMBs).

Methods: The pro-form of recombinant Der f 1 (rDer f 1) was produced using yeast expression system. Transform to active form of rDer f 1 was perfomed by partial mutation and sequential dialysis with acetate buffer. Proteolytic activity and substrate specificity of rDer f 1 were compared with those of its native counterparts. BMBs grown in culture were stimulated with active or heat-inactivated rDer f 1. Production of IL-4 and IL-13 was measured by flow cytometry.

Results: Proteolytic activity of active rDer f 1 was stronger than its pro-form and inactivated form. BMBs were detected by surface staining from mouse bone marrow culture. Production of IL-4 was elevated at 18 hours after the stimulation by active rDer f 1 in BMBs. The mean fluorescence index (MFI) of IL-4 production by active rDer f 1 was two times greater than that by control. Furthermore, IL-13 production was also significantly increased by the stimulation of the active rDer f 1. Heat-inactivated rDer f 1 induced neither IL-4 nor IL-13.

Conclusion: These results suggest that proteolytic activity of rDerf 1 is required for induction of IL-4 and IL-13 in BMBs. Further studies on the active Der f 1-initiated signaling in innate immune system are necessary.

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The roles of phosphoinositide 3-kinase gamma for human eosinophil functions

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Background: Phosphoinositide 3-kinases (PI3Ks) are known to play a prominent role in fundamental cellular responses of various inflammatory cells, including proliferation, differentiation, and cell migration. PI3Ks can be divided into plural classes, and PI3K gamma is, in particular, known to be involved in cellular migration. We have already reported that PI3Kgamma is involved in the pathogenesis of asthma through the modulation of effector phase of asthma using PI3K gamma deficient mice. Eosinophils are thought to be involved in the pathogenesis of asthma, especially in effector phase. In this study, therefore, we investigate the role of PI3K gamma for human eosinophils function including migration, degranulation and adhesive capacity.

Method: Human eosinophils were isolated by CD16 negative selection method. Purified eosinophils were stimulated with selective PI3K gamma inhibitor, and chemotaxis for eotaxin was analyzed with Boyden chamber assay. And the levels of EDN (eosinophil-drived neurotoxin) in the supernatant in which eosinophils were stimulated by 20nM eotaxin for 4 hours were measured by ELISA. In addition, after treatment of eosinophils with PI3K gamma inhibitor for 1 h, cells were stimulated by eotaxin for 1 hours. The adhesive capacity of eosinophils to recombinant soluble inter cellular adhesion molecule-1 (ICAM-1) –coated plate was examined.

Results: As a result, selective PI3K gamma inhibitor suppressed eotaxin-induced eosinophil migration in a dose dependent manner through the inhibition of ERK 1/2 phosphorylation. And, eotaxin-induced EDN release was also significantly inhibited by PI3K gamma inhibitor. In addition, eotaxin-induced adhesive capacity to ICAM-1 –coated plate was significantly inhibited by selective PI3K gamma inhibitor.

Conclusion: These results indicate that PI3K gamma might be involved in human eosinophil function, and PI3K gamma may be a new therapeutic target of asthma.

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Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion competent eosinophil granules

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Background: Eosinophils may release their granule proteins extracellularly through exocytosis, piecemeal degranulation or cytolytic degranulation. The findings in diverse human eosinophilic disorders of both intact isolated eosinophil granules and clusters of free, extracellular eosinophil granules (Cfegs) indicate that eosinophil cytolysis occurs ***in vivo***; but mechanisms and consequences of lytic eosinophil degranulation are poorly understood. Defining the means by which eosinophils may release extracellularly intact granules is more cogent with our recent findings that isolated eosinophil granules remain secretion competent.[1] Recently, an active form of cell death, namely extracellular DNA trap cell death (also called ETosis), has been recognized in neutrophils and mast cells.[2]

Method: Eosinophils were purified from donor blood by negative selection. Cells were stimulated with the calcium ionophore A23187, phorbol myristate acetate (PMA), and immobilized IgG. The morphologies of cells and free granules were studied using electron microscopy and fluorescence or time-lapse microscopy. Characteristics of cell free-granules were studied by flow cytometry. Response to cell-free granules to eotaxin-1 (CCL11) were assessed by ECP release and time-lapse fluorescence microscopy of release of acridine orange from within granules derived from ETosis cells.

Results: Eosinophils, in response to A23187, PMA, and immobilized IgG, undergo extracellular DNA trap cell death (ETosis) that releases free eosinophil granules. Eosinophil ETosis (EETosis) develops rapidly (30 – 120 min) in a NADPH oxidase-dependent manner. Initially, nuclear lobular formation was lost and some granules are released by budding off from the cell as plasma membrane-enveloped clusters. Following cytoplasmic chromatolysis, plasma membrane lysis releases DNA that forms web-like extracellular DNA nets and releases non-plasma membrane bound, free intact granules. EETosis-released free eosinophil granules, still retaining eosinophil cationic granule proteins, were activated to secrete when stimulated with CCL11.

Conclusions: Our results indicate that an active NADPH oxidase-dependent mechanism of cytolytic, non-apoptotic eosinophil death initiates nuclear chromatolysis that eventuates in the release of intact secretion competent granules and the formation of extracellular DNA nets.

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MicroRNA-155 is essential for Th2 mediated allergen-induced eosinophilic inflammation in the lung

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Background: MicroRNAs (miR) are a growing class of small noncoding RNAs that regulate gene expression by targeting mRNAs. Thus, these molecules are emerging as important modulators in cellular immune responses. The aim of the present study was to determine the role of microRNA-155 (miR-155) *in vivo* in a mouse model of allergen-induced eosinophilic airway inflammation.

Methods: Wild type mice (WT) and miR-155 knockout (KO) mice were sensitized and then challenged to allergen (OVA) to induce allergic airway inflammation. Eosinophilic inflammation was determined by differential cell count and T cell subsets ($(CD4^+GATA3^+$ (Th2 cells) and $CD4^+ROR\gamma^+$ T cells (Th17 cells)) were evaluated by flow cytometric analysis. Chemokine expression was evaluated by ELISA method.

Results: No differences were found in allergen-induced bone marrow eosinophilia between WT and miR-155 KO mice. In contrast, allergen challenged miR-155 KO mice showed a significantly reduced number of eosinophils and neutrophils in the airways compared to allergen challenged WT mice. This decrease was reflected by a significant reduction in Th2 cells in allergen challenged miR-155 KO mice. In addition, the number of airway Th17 cells showed a tendency to decrease in allergen exposed miR-155 KO mice compared to WT mice. Furthermore, a significant decrease in both eotaxin-1 and -2 levels was found in the airways of allergen challenged miR-155 KO mice compared to allergen challenged WT mice.

Conclusions: Our data provides evidence that miR-155 is involved in the regulation of allergen-induced eosinophilic airway inflammation, possibly through the regulation of Th2 cells in the airways. Thus, miR-155 may have potential as a novel anti-inflammatory target for allergic airway inflammation.

Oral Abstracts Session 6

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Dendritic Cell-Dependent Epigenetic Modifications in Foxp3 and IL-10 on Regulatory T cells during Immunotherapy

Garcia MA and Nadeau KC

Division of Immunology and Allergy, Stanford University, 2012

Background: We have conducted food oral immunotherapy (OIT) clinical studies over several years at Stanford University. Throughout therapy, our published data suggest that improving clinical reactivity to food allergens is linked to specific molecular changes in epigenetics of Foxp3, increased gene expression, and enhanced function of regulatory T cells (Treg).

Recently, we and others have established a link between reduced methylation of CpG islands on the Foxp3 locus and conversion of effector T cells (CD4+CD25neg) into Treg (CD4+CD25hi). Additionally, a subset of dendritic (regulatory DCs) that have been implicated in promoting tolerance through induction of Treg from Teff cells.

We hypothesized that increased increased Treg numbers and suppressive activity during OIT was due to epigenetic modifications of 22 CpG islands (promoter and intron regions) and that this was dependent on interactions with dendritic cells. In this project, we evaluated whether the molecular changes in T cells of subjects undergoing OIT could be attributed to a subclass of dendritic cells, termed "regulatory DCs".

Methods: DCs and Teff cells were isolated from PBMCs of OIT patients (at baseline and on therapy) through magnetic bead separation. After co-culturing DCs with autologous Teff cells at a ratio of 1:3 for 3 days, the samples underwent pyrosequencing to determine CpG methylation in Foxp3 loci.

Results: There was a higher percentage of methylated CpG in Foxp3 loci from baseline DC-T cell cultures [87%+/- 3%] when compared to post-therapy co-cultures [56%+/- 7%] ($p = 0.01$). The greatest difference existed between pre-therapy [87+/- 3%] and 3-5 months post-therapy [45+/- 5%] ($p<0.01$). Interestingly, the percentage of methylated CpG in Foxp3 loci began to increase from 3-5 months post therapy [45+/- 5%] to 6-12 months after therapy began [63% +/- 9%] ($p<0.05$).

Conclusions: These data suggest that as subjects continue on a standard oral immunotherapy regimen, DCs play a role mediating demethylation of Foxp3 loci in Teff cells, thus allowing them to convert to Treg. This demethylation may account for increased Foxp3 transcription, greater Treg activity and consequently, improving clinical response to administered allergens. Further research will help explain whether these regulatory DCs contribute towards long term sustainable tolerance in patients undergoing oral immunotherapy.

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Direct recruitment of pro-inflammatory slan-dendritic cells by immune complexes in a model of allergic vasculitis

Knut Schaekel, Thomas Döbel, Anke Lonsdorf, Alexander Enk

Background: The recognition of immune complexes (ICs) by immune cells is regarded as the initial pathogenic event in allergic vasculitis. Dendritic cells (DCs) are critical in the orchestration of local inflammatory responses. We therefore asked whether DCs can be directly recruited to local ICs in a model of physiologically relevant fluid shear stress. In these studies we focussed on the population of slan (6-sulfo LacNAC)-DCs, a human blood and skin DC subset characterized by pronounced expression of the low affinity Fc gamma receptor III (CD16). SlanDCs are highly pro-inflammatory and serve as a major and early source of IL-12 and TNF-a and were recently identified as inflammatory dermal dendritic cells in psoriasis (Hänsel et al. Journal of Allergy and Clinical Immunology 2011).

Methods: We applied a flow chamber adhesion assay with and without endothelial cells to measure the arrest function of different DC subtypes to ICs.

Results: We could show that small glass surface-coated ICs alone are highly efficient in mediating the arrest of slanDCs under conditions that match venous blood flow. This IC mediated arrest was specific for slanDCs and was not found for $CD1c^+$ DCs and plasmacytoid DCs. By specifically blocking the Fc gamma receptors of slanDCs, we were able to show that adherence to immobilized ICs is dependent on CD16. In contrast, blocking of CD32 (Fc gamma RII) only slightly inhibited the adherence of slanDCs. In line with this, blocking of both CD16 and CD32 only marginally increased inhibition of DC arrest compared to blocking of CD16 alone. To confirm and extend these findings, we employed a second model. Here, microslides were seeded with human endothelial cells and incubated with anti-endothelial IgG antibodies prior to measuring the arrest function of DCs under physiologic shear stress. These studies confirmed our hypothesis that slanDCs can be recruited under physiologic shear stress to antibodies or ICs deposited on endothelial cells. Furthermore, slanDCs were frequently found by immunohistochemistry in early skin lesions of allergic vasculitis.

Conclusions: Taken together we show that under defined shear stress conditions immobilized ICs recruit in a CD16-dependent fashion a highly pro-inflammatory slan-expressing human DC subtype. Therefore, our data provide first evidence for slanDCs as being a cell type that meets important functional criteria for an involvement in the initiation of IC-mediated vasculitis.

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MicroRNA dysregulation in alveolar macrophages and its relevance to defective innate immunity and disease severity in asthma

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Background: Treatment-resistant severe asthma (SA) is associated disease exacerbation in response to viral infection,

elevated TNF-alpha expression within the airways and abnormal airway microflora. As macrophages are critical to host defence we have investigated microRNA expression in alveolar macrophages from SA patients. MicroRNAs (miRs) are short (20-26 nucleotides) single stranded RNAs that regulate the expression of a broad number of target genes by binding to their 3' Untranslated Region (3'UTR) and directly blocking their translation or inducing degradation of mRNA.

Method: Alveolar macrophages (AM) were recovered by bronchoalveolar lavage from healthy controls (HC) and patients with asthma, including SA and microRNA expression evaluated by microarrays and qRT-PCR.

Results: Alveolar macrophages from asthmatics were found to significantly over express a range of miRs, including miR27, miR150, miR152, miR155 and miR375. All of these have seeding sites within the 3'UTRs of genes that lie within host defence pathways. MiR-150, miR-152 and miR-375, which were significantly increased in SA, putatively regulate TLR7 expression. Consistent with this we found TLR-7 mRNA and protein (Western blotting) to be significantly reduced in AM from volunteers with SA compared to HC ($p<0.003$). This was identified as functionally relevant, as AM from SA exhibited deficient interferon-beta (IFN-β) responses to stimulation with both Imiquimod (TLR7 agonist) and HRV16 in comparison to those from HC (each $p<0.05$). Importantly we were able to reverse this defect using an anti-microRNA oligonucleotide mix that blocked miR-150, miR-152 and miR-375, indicative of the relevance of these miRs. This innate recognition defect will be compounded, as MiR-27, miR-152 and miR155 are predicted to target Ripk1 (which inhibits TNF-α secretion). Consistent with this we found that transfection with a mix of oligonucleotide mimics for these 3 miRs enhanced the gene expression of TNF-α in alveolar macrophages after their exposure to HRV16. Additional results also suggest the relevance of miRs to altered AM bacterial recognition in asthma.

Conclusions: These studies, which are being extended into the understanding of the epigenetic regulation of miRs, thus raise the potential that therapeutic modulation of miRs may open up novel avenues for disease control in asthma and in particular SA.

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T cell derived microvesicles induce mast cells production of IL-24: a possible link to inflammatory skin diseases.

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Background: Close proximity between mast cells and T cells has been demonstrated in several T cell mediated inflammatory diseases including rheumatoid arthritis, and psoriasis. We have recently shown that microvesicles (MV) derived from activated T cells can stimulate human mast cells. This pattern of activation involved the MAPK system and resulted in degranulation and the release of several cytokines such as IL-8 and oncostatin M *(J.Immunol 2010; 185: 4206)*.

In order to characterize this novel pathway of mast cell activation, we analyzed the specific gene expression profiling by microarray analysis and identified that this stimulation lead to production of several cytokines and chemokines, here-to-fore unknown in mast cells, such as IL-24.

Methods: Gene expression profiling was done by means of microarray analysis, IL-24 expression was validated by real time RT-PCR, and the product was measured by ELISA in the LAD2 human mast cell line. The biological activity of IL-24 was verified by analyzing the phosphorylation state of STAT3 in human keratinocytes.

Results: Confocal and flow cytometric analysis with PKH67 labeled T cell derived MV revealed that these microvesicles internalized into the cells and that their interaction with mast cells is time dependent that reached maximal effect within 24 hr. The present study also demonstrates that mast cells stimulated with MV derived from activated T cells, but not by IgE cross-linking, expressed and released IL-24. IL-24, also known as MDA-7, is a member of the IL-10 family that has been shown to be up-regulated in psoriatic skin. It was shown previously, that IL-24 induces the activation and phosphorylation of STAT3 in keratinocytes of human psoriatic lesion and from HaCaT human keratinocytes cell line. Indeed, activation of HaCaT cell line with supernatants derived from MV-activated mast cells lead to phospharylation of STAT3.

Conclusion: This is the first demonstration of IL-24 in mast cells. IL-24 is specifically produced by human mast cells activated by T cell derived MV but not on IgE cross linking. The production of IL-24 may represent a link between T cell-induced mast cell activation and the development of skin diseases.

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Differential responses of human mast cells following dengue virus reovirus and KSHV infections

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Background: Mast cells are important sentinel cells in host defence. However, the responses of mast cells to viral infection are poorly understood.

Method: Using primary cultured cord blood derived human mast cells as well as the HMC-1 mast cell line we compared the chemokine and type 1 interferon responses of mast cells to three human viral infections, Dengue virus (in the presence of dengue specific antibodies), Reovirus (Dearing type 3) and Kaposi's sarcoma herpes virus.

Results: Evidence of active virus infection and viral replication was obtained in response to reovirus and Dengue virus but only latent infection, evaluated by LANA staining, was observed in response to KSHV infection. Examination of chemokine responses demonstrated that Dengue virus in the presence of dengue specific antibodies and reovirus infections induced the production of type 1 interferons, CCL3, CCL4, CCL5 and CXCL10. CXCL8 production was strongly enhanced by reovirus infection. Both of

these viral infections were associated with the up-regulation of a number of IFN response genes, including ISG56 and MX1. Dengue virus also enhanced expression of viral RNA sensors including RIG-I and MDA5. Treatment of mast cells with exogenous type 1 interferons did not induce a similar chemokine profile. In contrast to reovirus and dengue virus, KSHV infection led to a highly selective CCL4 response from mast cells that was not associated with up-regulation of IFN response genes. Mast cell degranulation was not observed in response to any of these infections, under the conditions we employed.

Conclusions: CXCL8 and CCR5 ligands have been shown to have a critical role in human mast cell mediated recruitment of NK cells and CD56+ve T cells respectively (Burke et al. Blood 2008, McAlpine et al. FASEB J 2012) the distinct mast cell mediator responses to different types of viral infections may play a key role in dictating the nature and effectiveness of the ensuing immune response.

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The transcription factor TFE3 is a major regulator of mast cell mediated allergic response

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In the present work, the role of TFE3, a member of the MiT family of proteins, was explored. No change in mast cell number was observed in the skin, lung and peritoneal cavity of TFE3 knockout mice when compared to WT mice. However, a substantial decrease was observed in the levels of c-KIT and FcεRIα expression in both peritoneal mast cells and in cultured mast cells derived from TFE3 knockout mice. This was followed by a significant reduction in the ability of TFE3 (-/-) mast cells to degranulate and to secrete cytokines. Furthermore, the level of histamine in the blood of TFE3 knockout mice following allergic trigger was substantially less than that of WT mice (1). The fact that TFE3 is emerging as a major transcription factor in the regulation of mast cell activity has great significance for the field as it provides direct evidence for another target which could be regulated in mast cell associated diseases.

We have previously reported that PIAS3 functions *in vivo* as a key molecule in suppressing the transcriptional activity of both MITF and STAT3 and defined the specific sequence that allows PIAS3 to bind to MITF as a short domain ($PIAS3_{82-132}$: spanning residues 82-132 out of 628 residues) (2).

By using Crustal software (http://www.ebi.ac.uk/Tools/msa/clustalw2/) we found high homology in the leucine zipper domain that is bound by PIAS3 in MITF and TFE3. We therefore have identified PIAS3 as a novel endogenous inhibitor for TFE3 and also made significant progress showing that a fragment of PIAS3, $PIAS3_{82-132}$, binds to TFE3 and is sufficient to inhibit its transcriptional activity.

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Mast cells and vitamin D_3: A 'D'-lightful regulatory interaction

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Mast cells (MCs) have long been recognized as active participants of the allergic response at specific sites. Whether in the skin, lung or gut, the binding and cross-linking of IgE on the surface of MCs stimulates the release of inflammatory mediators that exacerbate the allergic response. However, there is emerging evidence that in certain immune settings MCs have the potential to function as negative regulators of inflammation. Recently, we reported that biologically active vitamin D_3, 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), can induce IL-10 production *in vitro* from mouse bone marrow-derived cultured MCs (BMCMCs). Additionally, we demonstrated that MC-vitamin D receptor (VDR) expression was essential to ensure optimal MC-dependent protection against detrimental inflammation and pathology associated with chronic ultraviolet-B irradiation of the skin in $Kit^{W/W-v}$ mice. Based on these findings, we investigated if the well-recognised pro-inflammatory properties of MCs in IgE-driven immune settings can be reduced upon administration of biologically active vitamin D_3 ($1\alpha,25(OH)_2D_3$).

In a VDR-dependent manner, IgE + specific antigen-stimulated BMCMCs exhibited a reduction of histamine and β-hexosaminidase release, as well as decreased production of cysteinyl leukotrienes and the pro-inflammatory cytokines, TNF and IL-6, in response to the presence of exogenous $1\alpha,25(OH)_2D_3$ *in vitro*. To elucidate the roles of $1\alpha,25(OH)_2D_3$ and MC-VDR expression in a model of IgE-mediated MC-dependent passive cutaneous anaphylaxis (PCA) *in vivo*, we selectively engrafted the skin of two different genetically MC-deficient mouse strains, C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ and $WBB6F_1$-$Kit^{W/W-v}$ mice, with BMCMCs obtained from C57BL/6 wild-type or $VDR^{-/-}$ mice. We found that topical application of $1\alpha,25(OH)_2D_3$ significantly curtailed the magnitude of PCA-associated ear swelling. Notably, this effect required the presence of dermal MCs and their expression of VDR.

Taken together, these findings provide evidence that 1α,25(OH)$_2$D$_3$ can suppress IgE-mediated MC activation in a VDR-dependent manner both *in vitro* and *in vivo*. Ultimately, 1α,25(OH)$_2$D$_3$ might have therapeutic potential for the treatment and/or prevention of MC-dependent IgE-associated allergic disorders.

Oral Abstracts Session 7

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Mast cell activation is suppressed following co-culture with differentiated primary bronchial epithelial cells

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Background: Mast cells (MCs) are tissue-resident immune cells which lie in close proximity to the bronchial epithelium in asthma. Bronchial epithelial cells (BECs) are the structural cells of the airway that form a physical barrier against components of the inhaled environment and are an integral component of the innate immune system. Recent evidence suggests that there is cross-talk between BECs and MCs in asthma and therefore we hypothesized that BECs can influence MC function.

Methods: To determine the effect of a fully differentiated epithelium on MC function, BECs were differentiated at an air-liquid interface on a nanoporous membrane insert for 21 days, the human MC line, LAD2, were added to the basal compartment, IgE added and the cells cultured for 24h. The influence of differentiated BECs on LAD2 degranulation was assessed by β-hexosaminidase (β-hex) release following anti-IgE or ionomycin stimulation. Expression of CD117 and FcεRI by LAD2 cells was assessed by flow cytometry. The effect of LAD2 on epithelial barrier function was assessed by measurement of trans-epithelial resistance (TER).

Results: Co-culture of LAD2 cells with differentiated BECs resulted in a statistically significant reduction in β-hex release following activation with anti-IgE compared to LAD2 cells alone while β-hex release in response to ionomycin remained unchanged. The reduction in response to anti-IgE could not be explained by alterations in receptor expression since CD117 and FcεRI levels remained unchanged. There was no effect of LAD2 cells on the barrier properties of BECs (TER). We next attempted to determine the mediator from BECs responsible for the suppression of MC activation. We measured TGF-β2 by ELISA and observed an increase in the co-culture compared to either cell type alone. However blockade of TGF-β2 using blocking antibodies had no effect on the inhibition of MC activation by BECs. We are currently exploring other candidates which may be released from BECs which have the potential to suppress MC activation.

Conclusion: Differentiated BECs suppress MC activation. This regulatory function may be dysregulated in asthma.

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Insights into FcεRI signal initiation through defined valency ligands

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Background: Multivalent antigens trigger robust FcεRI-dependent signaling responses in mast cells and basophils. However, the chemical heterogeneity of most reagents render it difficult to predict receptor cluster size and orientation.

Methods: This work describes design of a trivalent ligand, comprised of fibritin trimer with DNP hapten attached to its amino-terminus by a short flexible linker. *In silico* docking and computer simulation methods were used to build structural models of ligand-IgE-FcεRIα ectodomain complexes and to estimate distances between individual receptors in aggregates.

Results: DNP$_3$-Fibritin stimulates degranulation and calcium responses in a dose-dependent manner, with maximal secretion at 10 nM. DNP$_3$-Fibritin is inhibitory at high doses, exhibiting a bell-shaped secretory response. Receptor aggregates persist at concentrations as high as 300 nM, interpreted as evidence for active inhibitory signals rather than a predominance of monovalent interactions.

Conclusions: Structurally defined antigens provide an improved basis for understanding FcεRI signal initiation and termination. These studies lay the foundation for understanding the essential structural features in natural allergens, such as valency, epitope distance and affinity.

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Activation phenotypes are enhanced in interacting mast cells and eosinophils: Functional implications of the allergic effector unit

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Background: Mast cells and eosinophils, the key effector cells in allergy, are abundantly co-localized particularly in the late and chronic stages of allergic inflammation. Recent evidence has outlined a specialized "allergic effector unit" (AEU) in which mast cells and eosinophils communicate via both soluble mediators and physical contact. In particular, a direct interaction between the co-stimulatory CD2 family molecules, CD48 on mast cells and 2B4 on eosinophils, was demonstrated to take place in vitro and in the allergic tissue. However, the functional impact of this bi-directional crosstalk on the cells' effector activities has not been revealed as yet. We therefore aimed to investigate whether mast cell/eosinophil interactions can influence the immediate and late activation phenotypes of these cells.

Methods: Human or murine mast cells and eosinophils were co-cultured under various conditions for a few hours or 1-3 days, and in selected experiments cell-cell contact was blocked. Cell migration and mediator release were examined, and flow

cytometry was applied to stain intracellular signaling molecules and surface receptors.

Results: Eosinophils enhanced basal mast cell mediator release and co-stimulated IgE-activated mast cells in a cell-cell ratio-dependent manner. This increased activation required physical contact through CD48-2B4 interactions, as neutralizing antibodies for these molecules abrogated the effect. Similarly, mast cells from CD48-lacking mice showed lower stimulation by wild-type derived eosinophils, and eosinophils from 2B4-lacking mice were less capable of activating wild-type derived mast cells. On the other end, resting and IgE-stimulated mast cells led to enhanced eosinophil migration, as well as to eosinophil activation through a mechanism that did not require physical cells' interaction. Moreover, increased phosphorylation of activation-associated signaling molecules, and enhanced release of tumor necrosis factor (TNF)-α, were observed in long-term co-cultures. Eosinophils also showed enhanced expression of intercellular adhesion molecule (ICAM)-1, which depended on direct contact with mast cells.

Conclusions: Our findings reveal a new role for the AEU in augmenting short and long-term activation in both mast cells and eosinophils, in a combined physical/paracrine manner partly involving CD48-2B4 interactions. This enhanced functional activity may thus critically contribute to the perpetuation of the inflammatory response in allergic conditions.

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Alternative mechanisms of eosinophilopoiesis: environmental factors influence neonatal immunity through alteration of cord blood progenitor CD34⁺ cell responses

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Rationale: Experimental and epidemiological evidence supports the role of maternal environmental exposures in neonatal immunity. In fact, genetic and environmental factors may lead to the development of allergies through effects on immune development during fetal life and early childhood. We have repeatedly shown that hematopoietic processes responsible for adult allergic inflammatory responses are also evident in infant cord blood (CB). Since environmental exposures in early life are important to the development of allergies, and given recent evidence which supports a role for Toll-like receptors (TLR) and thymic stromal lymphopoietin (TSLP) in myeloid differentiation, we investigated the role of these innate immune stimuli on CB progenitor cell differentiation in relation to infant allergic risk.

Methods: Human CB CD34⁺ progenitors were cultured in methylcellulose for hematopoietic cytokine-stimulated eosinophil-basophil (Eo/B) colony forming units (CFU), with or without lipopolysaccharide (LPS) or TSLP. Additionally, LPS-stimulated CD34⁺ cells were assessed for cytokine secretion using Luminex or signaling molecule activation using flow cytometry. Pharmacological inhibitors were added to cultured CD34⁺ cells to assess the roles of signaling molecules in Eo/B CFU and cytokine secretion. In a small allergy cohort, thirty-two infant CB samples were cultured in methylcellulose assays with LPS to assess alterations in Eo/B differentiation.

Results: LPS- or TSLP-stimulated CD34⁺ cells produce more GM-CSF- or IL-5-responsive Eo/B CFU, respectively. Increased secretion of GM-CSF after LPS stimulation serves as an autocrine signal to induce Eo/B CFU formation *ex vivo*. CD34⁺ cells respond to LPS stimulation through the time-dependent up-regulation of p38 MAPK, which is involved in GM-CSF secretion and GM-CSF-responsive Eo/B CFU formation. When infant allergic risk was assessed, LPS stimulation of high-atopic risk progenitors reduced GM-CSF-responsive Eo/B CFUs.

Conclusions: We show for the first time that LPS or TSLP can induce Eo/B CFUs, with the former operating via an autocrine mechanism of p38 MAPK-dependent GM-CSF secretion. Maternal allergy combined with microbial exposure in early life modulates hematopoietic progenitor differentiation *in utero* by circumventing the production of pro-allergic cells at birth. These alternative mechanisms of eosinophilopoiesis may shed light on possible targets for therapeutic interventions for allergic disease in early life.

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Differing mechanisms for Siglec-F- and Siglec-8-induced eosinophil apoptosis

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Background: Siglec-F and Siglec-8 are functional paralog proapoptotic cell surface receptors expressed on mouse and human eosinophils, respectively. Whereas Siglec-8 mediated death involves caspases and/or reactive oxygen species (ROS) generation and mitochondrial injury, very little is known about Siglec-F-mediated signaling and apoptosis. Therefore, the goal of this paper was to better define apoptosis pathways mediated by Siglec-F and Siglec-8. Given that Siglec-F-induced apoptosis is much less robust than Siglec-8-induced apoptosis, we hypothesized that mechanisms involved in cell death via these receptors would differ.

Method: Consequences of activation via Siglec-8 on human eosinophils and Siglec-F on mouse eosinophils was studied by measuring ROS production, and by performing apoptosis assays using eosinophils from normal, hypereosinophilic, NADPH oxidase deficient and src homology domain-containing protein tyrosine phosphatase (SHP)-1 deficient mice. Inhibitors of caspase activity and phosphoinositide (PI) 3 kinase activity were also used.

Results: Engagement of Siglec-F induced mouse eosinophil apoptosis that was dependent on caspase activity. There was no detectable ROS generation, nor any role for NADPH oxidase or SHP-1 in this apoptotic process. In contrast, human eosinophils

generated a robust ROS response following Siglec-8 engagement and ROS production and subsequent apoptosis were completely blocked by the PI3 kinase inhibitors LY294002 and Wortmannin.

Conclusions: These data suggest that Siglec-F-dependent apoptosis is predominantly caspase-dependent, while Siglec-8-mediated apoptosis occurs predominantly via PI3 kinases and ROS generation. One implication of this work is that mouse models targeting Siglec-F may not provide identical mechanistic predictions for consequences of Siglec-8 targeting.

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Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps required for efficient killing of Staphylococcus epidermidis

Dagmar Simon, Mahbubul Morshed, Shida Yousefi, Christina Stöckle, Hans-Uwe Simon

Background: Thymic stromal lymphopoietin (TSLP) that is released by epithelial cells upon certain environmental triggers activates cells of the innate and adaptive immune system resulting in a preferential T helper 2 immune response. By releasing eosinophil extracellular traps (EETs), eosinophils achieve an efficient extracellular bacterial killing. EET release, however, has been observed both in infectious and non-infectious eosinophilic diseases.

Objective: To investigate whether eosinophils generate functional EETs as a direct response to TSLP, and further to study the extra- and intra-cellular mechanisms involved in this process as well as TSLP receptor (TSLPR) expression by eosinophils in vitro and in vivo.

Methods: TSLPR expression on blood and tissue eosinophils was assessed by immunoblotting, flow cytometry and immunofluorescence staining. Purified eosinophils were stimulated with recombinant human TSLP. The release of extracellular DNA in association with eosinophilic cationic protein (ECP) was detected by fluorescence staining techniques and confocal microscopy. In addition, cell survival, cell adhesion, production of reactive oxygen species (ROS), and the inhibition of bacterial growth by TSLP-stimulated eosinophils were measured.

Results: TSLPR was observed on peripheral blood eosinophils as well as on tissue infiltrating eosinophils in skin diseases. TSLP did not affect eosinophil survival, but induced the formation of EETs consisting of mitochondrial DNA in association with ECP in a concentration- and time-dependent manner. EET release could be inhibited either by blocking cell adhesion or ROS production. While eosinophils prevented growth of both *Staphylococcus aureus* and *Staphylococcus epidermidis*, the latter were unable to elicit EET formation and eosinophils required additional TSLP stimulation to achieve this antibacterial activity.

Conclusions: TSLP directly stimulates eosinophils to produce EETs. Our observations link epithelial TSLP expression triggered by environmental factors with pathogen defense mechanisms involving eosinophils.

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Regulated formation and functions of eosinophil lipid bodies in allergy and innate immune response to infection

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Background: Increased lipid accumulation within cytoplasmic lipid bodies (also known as lipid droplets) in leukocytes are commonly observed pathological features of a number of infectious, allergic, neoplastic and other inflammatory conditions. We investigated the mechanisms involved in eosinophil lipid body formation and function in allergic and infectious diseases.

Methods and Results: By using a model of allergic pulmonary inflammation, we observed that allergen challenge-elicited lipid body biogenesis, and leukotriene (LT) C_4 synthesis were markedly dependent of the cooperation of MIF and eotaxin acting in a positive-feedback loop, via cross-talk between CD74 and CCR3. *Mycobacterium bovis* BCG or *Schistosoma mansoni* infection in mice also triggered eosinophil lipid body formation. Formation of eosinophil lipid bodies triggered by either BCG or S. mansoni infection was significantly reduced in Toll-like receptor-2 (TLR2)-deficient mice compared to wild-type mice, suggesting a role for pattern recognition receptors in lipid body formation induced by infection. Enzymes involved in eicosanoid synthesis were shown to localize at lipid bodies and direct localization of sites of eicosanoid synthesis by EicosaCell technique identified lipid bodies as major sites for eicosanoid generation in eosinophils following allergic or infection conditions. Of note, the compartmentalization of eicosanoid-forming enzymes and the eicosanoids generated within lipid bodies varied upon the stimulatory condition, providing evidence of functional heterogeneity of lipid bodies.

Conclusions: The mechanisms that govern lipid body biogenesis are highly regulated, and involve stimulus-dependent pathways. In eosinophils lipid bodies are inducible organelles with major functions in cell signaling and control of the synthesis and secretion of inflammatory mediators and attractive targets for novel anti-inflammatory and anti-allergic therapies.

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Functional aggregation of major basic protein in eosinophil granules and inflamed tissues

Hans-Uwe Simon, Alice Soragni

Eosinophils play important roles in innate immunity and contribute to immunopathology in various inflammatory and neoplastic disorders. In their specific secretory granules, they store several cytotoxic proteins. Currently, it remains largely unknown how the toxicity of these proteins is controlled within the eosinophil itself. Here, we show, with a combination of dye/immunostain and immuno-transmission electron microscopy and X-ray micro-crystallography, that eosinophil major basic protein (MBP), which is present in the core of specific granules, is packed into a crystal lattice with amyloid-like properties, enabling

the inert storage of the otherwise toxic protein. Moreover, we demonstrate that following eosinophil activation, MBP is mobilized, unpacked, and released as non-toxic oligomers. Upon secretion, an aggregation process is initiated, resulting in the generation of toxic MBP, which is then able to efficiently kill bacteria. Interestingly, we also detected larger extracellular non-toxic MBP aggregates in eosinophilic tissues, explaining, at least partially, how tissue destruction can be limited under such pathological conditions. Taken together, our evidence argues that the toxicity of eosinophil MBP is controlled by functional amyloid formation and that there exists only a narrow border between functional and disease-associated aggregation.

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Critical role of P1-Runx1 in mouse basophil development

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Background: The relationship between basophils and mast cells in hematopoiesis is still not entirely clear. Moreover, relatively little is known of the transcription factors which regulate basophil development at baseline or during induced basophilia. Runt-related transcription factor (Runx) proteins are a family of transcription factors which have crucial roles during the development of many tissues, and the immune system. Studies of conditional Runx1 knockout mice indicate that Runx1 can regulate differentiation of hematopoietic stem cells (HSCs), B-lymphocytes, NKT cells, and T-lymphocytes. All three Runx genes can be transcribed from the distal (P1) or proximal (P2) promotors, and M. Tachibana *et al.* (*J. Immunol.* 2010;186:1450) demonstrated a requirement for P1-Runx1 in lymphoid tissue inducer cell differentiation and found that *Runx1*$^{P1N/P1N}$ mice have severe reductions in NKT cells, mild T cell deficits, and an increase in Lin$^-$c-Kit$^+$Sca-1$^+$ HSCs. However, there have been no previous reports describing the myeloid cell compartment in these mice.

Methods: We analyzed basophil and mast cell development and functions in C57BL/6 wild type (WT) mice and in C57BL/6 *Runx1*$^{P1N/P1N}$ mice deficient in the transcription factor distal promoter-derived Runx1 (P1-Runx1).

Results: We found that *Runx1*$^{P1N/P1N}$ mice had a >90% reduction in the numbers of basophils in the bone marrow, spleen and blood. In contrast, *Runx1*$^{P1N/P1N}$ mice had normal numbers of the other granulocytes: neutrophils and eosinophils. Although basophils and mast cells share some common features, *Runx1*$^{P1N/P1N}$ mice had normal numbers of mast cells in multiple tissues. *Runx1*$^{P1N/P1N}$ mice failed to develop a basophil-dependent reaction, IgE-mediated chronic allergic inflammation of the skin, but responded normally when tested for IgE- and mast cell-dependent passive cutaneous anaphylaxis *in vivo* or IgE-dependent mast cell degulanulation *in vitro*. These results demonstrate that *Runx1*$^{P1N/P1N}$ mice exhibit markedly impaired function of basophils, but not mast cells. Infection with the parasite *Strongyloides venezuelensis* and injections of IL-3, each of which induced marked basophilia in WT mice, also induced modest expansions of the very small populations of basophils in *Runx1*$^{P1N/P1N}$ mice. Finally, *Runx1*$^{P1N/P1N}$ mice had normal numbers of the granulocyte progenitor cells, SN-Flk2$^{+/-}$, which can give rise to all granulocytes, but exhibited a >95% reduction in basophil progenitors (BaPs).

Conclusions: These observations suggest that P1-Runx1 is critical in mice for a stage of basophil development between SN-Flk2$^{+/-}$ cells and BaPs. They also provide another example of a difference in the regulation of basophil and mast cell development in mice.

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New-generation, taste-masked epinephrine sublingual tablets: Preclinical study

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Background: Epinephrine (E) is life-saving treatment in anaphylaxis. In community settings, a first-aid dose of E is injected into the mid-outer thigh from an epinephrine auto-injector. Patient concerns about E auto-injectors include: large bulky size, "getting a needle", availability of only two fixed epinephrine doses (0.15 mg or 0.3 mg), and a short shelf-life.

Previously, in a validated preclinical model, our first-generation E sublingual (SL) 40 mg tablet formulation resulted in serum E concentrations similar to ($p>0.05$) those achieved after E 0.3 mg IM injection (*J Allergy Clin Immunol 2006;117:398-403)*. These E SL 40 mg tablets have a 7 year shelf-life.

Methods: New-generation SL E 40 mg tablets were developed by modifying the non-medicinal ingredients (NMIs). By addition of citric acid, we masked the intrinsic bitter taste of epinephrine and by changing the proportions of mannitol, microcrystalline cellulose and a "super disintegrant", we reduced disintegration times to £ 13 seconds and decreased dissolution time for epinephrine to £ 60 seconds.

We studied the rate and extent of E absorption from these new-generation SL tablets in our rabbit model, using a crossover design. The positive control was E 0.3 mg IM from an EpiPen. The negative control was a SL tablet containing only the NMIs (no E). Tablets were placed under the tongue for 2 minutes. Blood samples were collected at frequent intervals through an in-dwelling arterial catheter. E concentrations were measured using HPLC with electrochemical detection. Serum concentration versus time data were analyzed using standard pharmacokinetic equations and the WinNonlin program.

Results: The mean ± SD maximum serum concentration (C_{max}) of 31.7±10.1 ng/mL at a peak time (T_{max}) of 20.0±7.1 min, and area

under the curve ($AUC_{0\text{-}1\ hr}$) of 678.0±149.0 ng/mL/min after SL E 40 mg did not differ significantly from the C_{max} of 27.6±7.0 ng/mL at a T_{max} of 30±0.0 min with an $AUC_{0\text{-}1\ hr}$ of 592.0±122.3 ng/mL/min after E 0.3 mg IM in the thigh (p>0.05).

Conclusion: E is absorbed rapidly from the new-generation, taste-masked, fast-disintegrating SL formulation. These tablets are suitable for Phase I studies in humans and might be useful in the first-aid treatment of anaphylaxis.

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Confirmation of the greatest sensibility and specificity of basophils autoinduced degranulation test in the diagnosis of chronic autoimmune urticaria

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Background: A rate of subjects (15-30%) affected by chronic idiopathic urticaria (CIU) have an autoimmune aetiology thus as to be classified as Chronic Autoimmune Urticaria (CAU). The diagnosis of CAU is based on the demonstration of the presence of anti-FcεRIα and/or anti-IgE autoantibodies in the serum. The commonly screening procedures is represented by Autologous Serum Skin Test (ASST), but this test has low sensitivity and specificity (70%-80%). The aim of this study is to demonstrate that the new ***in vitro*** method such as Basophils Autoinduced Degranulation Test (BADT) is useful and reliable to be at least associated to ASST in the diagnosis of CAU.

Method: 24 CAU affected patients, previously diagnosed by BADT were submitted to ASST in order to confirm the diagnosis. Serum and peripheral whole blood were collected before the ASST procedure. The serum was divided into two parts, one of which was decomplemented (DS), and the other was used whole (WS). These sera were employed to stimulate autologous peripheral whole blood *in vitro* and to perform skin test on subjects. In order to perform the BADT we used a two-colours protocol in flow cytometry with anti-human CCR3 and CD63 antibodies marked respectively by R-PhycoErythrin (PE) and Fluorescein IsoThioCyanate (FITC).

Results: All the subjects showed a significant increase of CD63 expression on basophils stimulated with WS and/or DS, but just 21 of 24 CIU patients showed a positivity to ASST. All the subjects were positive to the histamine control. Of the BADT 12 on 24 subjects showed degranulation only with WS, only 1 with DS and 11 with both stimuli.

Conclusions: BAD test is useful and more reliable of ASST for the determination of autoimmune etiopathogenesis of the CIU. Recent studies conducted with similar methods confirmed that the sensitivity and specificity of BAD test are approximately of 95% and 90%. In our study we confirmed 100% of the CAU diagnosis by BADT, and only 87,5% by ASST.

Moreover our method is more faster and cheaper than previous that used leucocytes separation and a three-colours procedures.

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Clinical features of contrast media anaphylaxis

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Background: As the use of iodine based contrast media is rapidly growing, the incidence of contrast media hypersensitivity including anaphylaxis is increased. However, the pathomechanism and clinical characteristics of contrast media hypersensitivity are not clearly defined. This study was performed to investigate the clinical features and risk factors of contrast media induced anaphylaxis.

Methods: We retrospectively reviewed electronic medical records of patients diagnosed as contrast media related anaphylaxis from January 2005 to March 2012 at Seoul National University Hospital, in Seoul, Korea and analyzed the data to identify risk factors of the development of anaphylaxis.

Results: A total of 94 cases were diagnosed as contrast media related anaphylaxis during study period. The mean age was 55.9±13.3 years and 38 (40.4%) of them were male. While the mean number of contrast exposure was 4.7 when the first anaphylaxis occurred, 35% of anaphylaxis developed at the first exposure to contrast media. Among the patients who had multiple contrast exposure before the development of anaphyalxis, 24.6% of them already experienced mild to moderate RCM hypersensitivity reaction, such as itching, urticaria, angioedema. Twenty six patients who had experienced anaphylaxis were reexposed to contrast media after premedication with chlorpheniramine or systemic steroid and hypersensitivity response reappear in 7 (26.9%) including 5 cases of anaphylaxis. Skin test with contrast media was performed in 29 patients and 18 (62.1%) showed positive results. Skin test to RCM showed high positive rate among patients with anaphylaxis (62.1%), especially in patients with anaphylactic shock (82.4%). Number of previous RCM exposure were significantly higher in patients with anaphylactic shock compared with normotensive anaphylaxis (6.0±7.6 vs. 2.8±3.5, p=0.009).

Conclusion: We found that 65% of anaphylaxis developed after multiple exposures to contrast media and mild reactions heralded anaphylaxis in 1/4 of patients who were repeatedly exposed to contrast media. RCM exposure was significantly more

frequent in patients with anaphylactic shock than in patients with normotensive anaphylaxis.

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Low proteolytic stability - one of the driving forces of allergenicity?

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Background: Recently, it became evident that factors activating the innate immunity play a central role in the development of allergic diseases. The major birch pollen allergen Bet v 1 was among the first allergens cloned and produced as recombinant proteins; still, the allergy-inducing mechanisms of Bet v 1 remain unclear. Phylogenetically, the birch pollen allergen belongs to the pathogenesis-related protein 10 family, a collection of a large number of generally non-allergenic proteins showing a distinct conserved three-dimensional structure. By comparing non-allergenic Bet v 1 homologues with the major birch pollen allergen, we wanted to identify elements within the Bet v 1 architecture, which account responsible its allergenicity.

Methods: Four non-allergenic structural homologues of Bet v 1 from plant, bacterial, and archaeal sources were identified, produced in *E. coli* and purified to homogeneity. The proteins were characterized using a full array of physico-chemical methods to investigate structural properties, stability, and aggregation behavior.

Results: One of these four Bet v 1-homologues Tth PR-10 originating from a thermophilic bacterium showed extraordinary heat-stability when compared to Bet v 1. The increased stability of Tth PR-10 directly translated into elevated resistance towards endo-/lysosomal proteolysis by dendritic cells (DC), which influenced antigen-presentation by these cells. Thus, the increased stability altered both immunogenicity and immune-polarizing properties of the molecule. In an adjuvant-free mouse immunization model, we were able to demonstrate that the immunogenicity towards Tth PR-10 was strongly increased compared to Bet v 1. Moreover, the proteolytically stable Tth PR-10 failed to induce a strong TH2 response as observed for Bet v 1 and antigen-specific IgE levels were significantly lower, both indicators for immune deviation towards TH1.

Conclusion: The data provide strong evidence that protein stability is an inherent structural property intimately connected to allergenicity. Low proteolytic stability and fast processing of protein antigens by DCs leading to an ineffective delivery of T cell-reactive peptide complexes to the cell surface can be considered as key event in the development of allergies. Understanding these parameters in combination with extrinsic allergy promoting factors will definitely guide the way for the development of novel allergy therapeutics.

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Dog saliva – an important source of dog allergens

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Background: Allergy to dog (*Canis familiaris*) is a worldwide common cause of asthma and allergic rhinitis. However, dog dander extract in routine diagnostics is not an optimal predictor of IgE-mediated allergy to dog. The aim of this study was to evaluate dog saliva as an allergen source for improved diagnosis of dog allergy.

Methods: IgE binding proteins in dog saliva and dander extract were analyzed by immunoblot and mass spectrometry (MALDI-TOF and LC-MS/MS) using pooled or individual sera from dog allergic patients (n=13). Sera from 59 patients IgE positive to dander (median 26 kU_A/L; range 1.22-100 kU_A/L) and 55 patients IgE negative to dander (<0.1 kU_A/L) but with symptoms to dog were analysed for IgE against saliva and dander by ELISA. IgE values to dog dander and saliva were considered positive in ELISA when the IgE responses exceeded mean + 3SD of the 67 non-dog sensitized controls (OD ≥ 0.085 for dog dander; OD ≥0.123 for dog saliva). Basophil stimulation with dog saliva and dander extract was measured by flow cytometry among three dog allergic patients. Additionally, IgE binding protein profiles of saliva from different breeds were investigated by immunoblot.

Results: A greater number and diversity of IgE binding proteins was found in saliva compared to dog dander extract and the amount varied among dog breeds. In saliva Can f 1, 2 and 3 were identified but also parotid secretory protein as a new saliva allergen candidate. The majority of the 59 dog dander positive sera (n=44) were IgE positive to dog saliva (OD; median 0.276, range 0.123-0.891). Among patients IgE negative to dander, but with symptoms to dog, 20% were IgE positive to saliva (OD; median 0.139, range 0.125-0.188). The biological activity of saliva was confirmed by basophil degranulation.

Conclusions: The study reports dog saliva as an important source of dog allergens. One-fifth of patients with symptoms to dog, but IgE negative to dog dander, were found to have IgE against dog saliva. The IgE binding protein profile of saliva from different breeds varies.

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Art v 3, the non-specific lipid transfer protein of mugwort pollen: Monoclonal antibodies to localize conformational human IgE epitopes on the crystal structure of an allergen

Gabriele Gadermaier, Eva Vevjar, Iris Magler, Michael Hauser, Marina Liso, Adriano Mari, Hans Brandstetter, Fatima Ferreira, PhD

Background: Art v 3, the non-specific LTP (nsLTP) of mugwort pollen was shown to be involved in IgE cross-reactivity with homologues from various plant food sources. Although playing an important role in pollen-food mediated allergy, structural data of allergenic pollen nsLTP are still missing. Thus we aimed to resolve the 3-D structure and conformational IgE-binding epitopes of Art v 3.

Methods: Purified recombinant Art v 3.0201 was obtained from heterologous expression in *E. coli*, and subjected to crystallization by sitting-drop vapour diffusion measured at the ID29-beamline of ESRF/Grenoble. The structure was solved by molecular replacement using 2ALG as template. Purified nsLTP from mugwort, ragweed, peach, celery stalks, apple, strawberry and hazelnut were tested for IgE-reactivity in ELISA using sera of 21 mugwort pollen allergic patients. Monoclonal antibodies (moAb) were obtained after immunizing mice with Art v 3.0201 and tested for binding specificity towards the panel of purified LTPs. To identify potential human IgE epitopes of Art v 3, cross-inhibition assays were performed using moAbs and patients' sera.

Results: The 3-D structure of Art v 3.0201 was solved at a resolution of 1.9Å, showing to consist of four alpha helices stabilized by four intramolecular disulfide bonds. A tunnel-like cavity was identified which is well accessible for interaction with lipids. Mugwort allergic patients showed a broad spectrum of reactivity towards the investigated LTPs; however Amb a 6, the nsLTP from ragweed, was recognized by only 57% of patients. Three murine moAb (IgG1) were obtained presenting different recognition patterns, one reacting mainly with an Art v 3-specific epitope, whereas the remaining two also recognized the nsLTPs from peach, apple, hazelnut or celery stalks. IgE reactivity to Art v 3 was considerably blocked by the presence of moAbs and demonstrated >70% inhibition for individual sera (median=85%) suggesting the identification of relevant allergenic epitopes.

Discussion: Art v 3 displays typical LTP structural elements which might explain the frequent cross-reactivity within this allergen family. The use of murine moAb which demonstrate a broad LTP-reactivity and overlap with human IgE epitopes will allow identification of relevant allergenic patches on the structure of Art v 3 and cross-reactive homologues.

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Cloning of cDNA and IgE Reactivity of α-amylase from house dust mite, dermatophagoides farinae

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House dust mite (HDM) is the most important inducer of allergy and asthma in the world. Mite group 4 allergen is an α-amylase, and it is one of HDM›s minor allergens. In this study, we cloned the full cDNA of *D. farinae* (Der f) α-amylase by RT-PCR and RACE-PCR. Cloned Der f α-amylase DNA contained the open reading frame of 1,578 bp which encodes 525 amino acids. The deduced amino acid sequence showed 88, 65 and 66% sequence identity with the Der p 4, Tyr p 4 and Blo t 4, respectively. Recombinant Der f α-amylase was produced in *Escherichia coli* Rosetta 2 (DE3) and purified by Ni-NTA agarose affinity chromatography from inclusion body lysate, and then investigated its IgE reactivity by ELISA. Recombinant Der f α-amylase was recognized by IgE antibodies from 15 of 100 (15%) *D. farinae*-sensitized allergy patient's sera tested. This protein could be a useful candidate for development of allergy diagnostic tool and the design of immunotherapeutics.

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Influence of temperature and buffer composition on the stability of house dust mite extract

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Background: Allergen extract of house dust mite (HDM, *Dermatophagoides pteronyssinus*) is widely utilized for allergy diagnosis and therapy. Allergens in extracts degrade and lose potency on storage. Therefore, it is necessary to investigate the optimal condition to increase the stability of allergen extract.

Method: The influence of 0.03% human serum albumin (HSA) and 50% glycerol on HDM extract, which is standardized in Korea, was evaluated at -20°C, 4°C and room temperature (RT) in order to investigate the optimal storage condition. The changes of protein concentration, Der p 1 content and allergenicity over 1 year were measured by Bradford assay, two-site ELISA and ELISA inhibition. IgE-immunoblotting was also performed to examine degradation of allergens.

Results: Protein concentrations decreased to 14%, 49% and 94% at RT, 4°C and -20°C, respectively when stored at the distilled water (DW). Overall allergenicity was kept 89.9% when extract was reconstituted in 50% glycerol solution and 93.1% in 50% glycerol

and 0.03% HSA, whereas it was decreased to 36.6% and 33.3% in DW or 0.03% HSA solution at RT. Allergenicity was kept 92.0% to 97.0% when stored at 4°C regardless of buffer condition. IgE-immunoblotting indicated that a 15 kDa allergen, possibly Der p 2, is the most important for allergenicity of the extract, followed by a 42 kDa allergen. Furthermore, a 42 kDa protein was found to be one of the important allergens for the stability of HDM extract, for it was shown to be more easily degraded at RT.

Conclusions: The most important factor to increase the shelf-life of HDM extract was shown to be storage temperature (4°C). Addition of 50% glycerol was found to be important in the storage buffer to keep the allergenicity.

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The IgE and IgG antibody binding profile of the known spectrum of protein cat allergens

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Background: It was considered important to determine the IgE and IgG allergen-binding profile of cat-allergic subjects to design strategies for immunotherapy with defined allergens.

Method: A quantitative binding assay was used to compare the IgE and IgG antibody responses to the protein allergens Fel d 1, 2, 3, 4, 7, and 8 plus the IgE binding proteins haptoglobin and S100A12.

Result: Fel d 1 accounted for greater than 50% of the anti-cat IgE antibodies for 58% of cat allergic subjects, that were defined by the development of symptoms on exposure to cats and by > 0.35IU/ml of anti cat IgE. It was however only the highest IgE binding protein for a further 3% of subjects. The remaining subjects usually had one of the lipocalin allergens Fel d 4 or 7 as their highest IgE binding specificity or the LPS-binding protein homologue Fel d 8 and interesting in most cases one of these allergens accounted for over 50% of the IgE binding. It was shown with Fel d 7 that allergen-IgE complexes of a non-Fel d 1 allergen bound CD23. High titres of IgG1 anti-Fel d 1 were found in allergic and non-allergic subjects whereas IgG4 anti Fel d 1 antibodies were found at lower titre and were more prevalent in allergic subjects. IgG antibodies to the other allergens were restricted to the IgG4 isotype and were less prevalent and of lower in titres than those to Fel d 1. They were also mainly found in allergic subjects.

Conclusions: Non-Fel d 1 allergens are frequently the highest IgE-binding proteins in cat allergy. Additionally the absence of high-titre IgG1 antibodies to these allergens in allergic and non-allergic subjects shows a different immunoregulation to Fel d 1 and that their disease-inducing potential might not be as affected by IgG.

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Three more case studies corroborate suspected lack of specific IgE in diclofenac hypersensitivity

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Background: Hypersensitivity to diclofenac (DF), a representative of non-steroidal anti-inflammatory drugs, is considered non-immune-mediated (Harrer, et al., PLoS One, 2010). However, certain severe adverse reactions to DF resemble immediate IgE-mediated hypersensitivity. Results of skin tests with DF are not reliable. The diagnostic gold standard is the oral provocation test (OPT). The objective of this work was to determine whether selective severe confirmed DF hypersensitivity is associated with IgE-mediated hypersensitivity mechanisms.

Materials and Methods: Three patients with clinical evidence for IgE-mediated reactions were delivered to the hospital. All had suffered from severe grade III systemic reactions including loss of consciousness within 10 min after intake of DF. Intradermal tests (IDT; all patients) and OPT (patient 2 and 3) were performed. DF and DF-5-hydroxy (DF5OH) metabolite were covalently conjugated to human serum albumin (HSA) and characterized by high performance size-exclusion chromatography. Using sera of DF-KLH-sensitized mice, both conjugates were tested in ELISA for DF-specific mouse-IgG binding. The two conjugates were used for analysis of serum IgE in all patients. In addition, basophils of patient 1 were investigated in BAT for activation marker expression after stimulating either with DF-HSA or DF5OH-HSA.

Results: All patients demonstrated positive IDT. Patient 1 developed systemic grade II reaction 15 min after IDT. Patient 2 and 3 demonstrated positive OPT. Analysis of conjugation degree showed that 10.7 molecules DF and 16.1 molecules DF5OH were bound per HSA. In the mouse immuno-assay both conjugates were able to bind DF-specific IgG. In the sera of the DF-hypersensitive patients IgE was below the detection limit using DF-HSA as well as DF5OH-HSA. No upregulation of CD63 surface expression on basophils of patient 1 was observed.

Conclusions: No IgE was detected in sera of the hypersensitive patients using conjugates with approved functionality and immunogenicity. This finding was confirmed by negative BAT of patient 1. Hence, no proof for IgE-mediated mechanisms was found, even though positive reactions in the skin tests and provocation tests were observed and even one patient demonstrated a rare systemic reaction during the skin test. This confirms previous findings of our group and leaves the pathomechanism of diclofenac hypersensitivity unresolved.

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Prevention of allopurinol induced severe cutaneous adverse reactions in patients with renal insufficiency by screening test for HLA-B*5801: prospective study

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Background: Although allopurinol is a very effective urate-lowering agent for complicated hyperuricemia, it can induce severe cutaneous adverse reactions (SCARs). According to previous our study, incidence of SCARs rose to 18% in HLA-B58 allele positive patients with renal insufficiency. This study was conducted to evaluate the usefulness of prevention of allopurinol induced SCARs in patients with renal insufficiency by screening test for HLA-B*5801.

Methods: With Institutional Review Board of Seoul National University Hospital, HLA typing was done as a genetic screening test in patients with chronic renal insufficiency who were ahead of administration of allopurinol. HLA-B*5801 negative patients were prescribed allopurinol once a day as usual. For high risk patients with HLA-B*5801, allopurinol was administered based on 30-day desensitization protocol. Hypersensitivity reactions and other adverse reactions were surveyed during first 3 months.

Results: A total of 209 patients were evaluated the HLA type and prescribed allopurinol and 8 were unable to complete the study because of other drug adverse reaction, follow up loss or withdrawing their consent. In 201 patients, male was 144 (71.6%) and mean age was 61.50±14.51 years. In 14 (7.0%) patients with HLA-B*5801, there was no SCARs and one patient (14.3%) experienced simple rash with desensitization protocol. In 185 (92.0%) patients without HLA-B*5801, 13 patient (7.0%) experienced hypersensitivity reactions to allopurinol. Most common symptom was pruritus (12, 6.49%), followed by rash (4.32%) and urticaria (1.08%).

Conclusion: Screening for HLA-B*5801 and administration of allopurinol based on desensitization protocol in HLA-B*5801 positive patients can be used for the prevention of allopurinol induced SCARs.

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Class 3 food allergy induced by contact sensitization of foods

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Recently, an important hypothesis is proposed, suggesting that percutaneous absorption of food allergen induces food allergy, while oral consumption of food allergen induces oral tolerance from the experimental and epidemiological data. We report 19 patients with the immediate food allergies such as oral allergy syndrome and food anaphylaxis, which are supposed to be induced by contact sensitization of food allergens to the skin from present history, in vivo skin prick testing and the measurement of specific IgE antibodies. These patients are classified to the 2 groups by etiological factor. 8 patients of the 1st group handle frequently foods with empty hands in the work and / or occupation, result in the induction of food allergies by percutaneous sensitization. 19 patients of the 2nd group use frequently cosmetics containing foods and/or their processed ones such as wheat gluten hydrolysate, result in the food allergies as well. The patients of the 1st group have atopic dermatitis and /or hand eczema in 75% (6/8). On the other hand, the patients of the 2nd group have atopic dermatitis in only about 30% (3/11) and have atopic disease in 55% (6/11) even when other atopic disease such as polinosis is added. However, it is very interesting that most patients (10/11) of the 2nd group have the history of use of the washing face soap containing wheat gluten hydrolysate. These results indicate that the following factors such as the skin barrier disturbance found in AD patients, use of washing face soap promoting it, the high allergenicity of food side like wheat gluten hydrolysate and the sensitivity of host side are required for the percutaneous sensitization of foods, resulting in food allergy.

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YKL-40 level in induced sputum is an acute phase biomarker in allergic airway inflammation

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Background: Serum level of chitinase-like protein such as YKL-40 of asthmatic patients was known to positively correlate with disease severity but controversy is remained yet. Allergen bronchial provocation test (ABPT) can induce allergic airway inflammation in atopic asthma. The aim of this study is to evaluate whether YKL-40 is induced by allergen induced airway allergic inflammation in asthma and evaluate its kinetics.

Methods: Total thirteen patients were enrolled from May to November in 2008. All of the patients underwent ABPT using Dermatophagoides farinae crude extract. Induced sputa (IS) and sera were collected for three times - 7 days before ABPT for baseline, immediate-post ABPT and 24 hours after ABPT. We examined cytology of IS and measured levels of YKL-40, interleukin (IL)-4, IL-5, IL-13, IL-33, TNF-α and eosinophilic cationic protein (ECP) in IS and/or sera.

Results: In cytological examination, total cell, eosinophil and neutrophil numbers were increased after ABPT with time dependent manner. YKL-40 level was increased after ABPT not in sera but in IS samples with statistical significance (p=0.011). IL-5 and ECP levels in IS were also increased followed by ABPT like YKL-40 level (p=0.011 for IL-5 and p=0.006 for ECP). YKL-40 level in IS was well correlated with ECP level in IS (ρ=0.584, p<0.001).

Conclusions: YKL-40 level was increased immediately in IS of asthmatics and sustained to 24 hours after ABPT. As the level of YKL-40 is well correlated with eosinophilic and neutrophilic inflammation in IS, we suggested that the YKL-40 may play pathophysiologic role in human atopic asthma.

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Discovery of sputum biomarker for neutrophilic inflammation in severe uncontrolled asthma

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Background: Severe uncontrolled asthma (UA) is a specific phenotype of asthma, and comprises about 5% of total asthma. Neutrophilic airway inflammation has been frequently observed in severe uncontrolled asthma. In addition to the increase in the number of neutrophils infiltrated into the airways, neutrophils is expected to functionally activate.

Objective: To identify sputum biomarkers of severe uncontrolled asthma with neutrophilic airway inflammation

Methods: Sputum were pooled from 5 severe uncontrolled asthmatics (UA) and from 10 controlled asthmatics (CA) having > 70% of neutrophils. Two-dimensional electrophoresis was adopted for differential display proteomics and candidate proteins were identified using MALDI-TOF MS analysis. S100 calcium binding protein A9 (S100A9) was identified using Western blot, and the levels were measured using ELISA in sputum from the asthmatics with various severity, chronic obstructive lung diseases (COPD), and normal controls.

Results: 14 protein spots showed differences in relative intensity between severe UA and CA. MALDI-TOF/TOF revealed increase in human neutrophil peptide 2, S100A9, alpha-amylase, neutrophil gelatinase-associated lipocalin, 4-aminobutyrate transaminase, and cystatin SA in UA compared to CA, while decrease in plunc precursor, complement C3 component, immunoglobulin heavy chain variable region, glial fibrillary acidic protein isoform 1, IgM kappa IIIb SON, MLL-AF4 der(11) fusion protein, cytokeratin 8, and recombinant IgG4 heavy chain. S100A9 was clearly demonstrated in neutrophilic sputum of severe UA than those of CA or eosinophlic sputum of severe UA on western blot. S100A9 levels were significantly increased in neutrophilic UA more than those of CA, eosinophilic UA and CA and COPD.

Conclusion: S100A9 in sputum could be a biomarker of neutrophilic inflammation of UA.

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Sputum interleukin 6: A biomarker for stratified therapy in treatment-resistant severe asthma

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Background: Interleukin 6 (IL-6) is a pleotropic cytokine that has relevance to chronic inflammatory diseases, in that it favours mononuclear cell accumulation at sites of injury, promotes angioproliferation, has anti-apoptotic function on T cells and is critical to Th-17 cell differentiation. The successful demonstration of disease modification with tocilizumab, a neutralising humanised antibody to the IL-6 receptor, in patients with rheumatoid arthritis (RA) has confirmed the central role of IL-6 in this disease. As there may be overlap between the pathology of RA and airway mucosal inflammation in treatment-resistant severe asthma, we have investigated the expression of IL-6 within the airways in asthma.

Method: Interleukin 6 concentrations have been measured by ELISA in induced sputum and serum samples from patients with asthma (mild and treatment-resistant severe asthma) as well as healthy controls. Sputum measures were made in both DTT and PBS processed sputum samples to evaluate the impact of sample processing and the IL-6 concentrations related to a range of parameters of asthma severity.

Results: The median IL-6 concentration in DTT processed induced sputum samples from healthy controls (n=17) was 9.46 pg/ml (95% CI 5.46 - 13.65 pg/ml). This was significantly (p=0.001) lower than that in asthma (n=48, median IL-6 26.7pg/ml). Of the 39 with severe asthma, 27 (69.2%) had sputum IL-6 concentrations outside the upper CI of that in healthy subjects. Measures in DTT processed samples were, however, found to be suboptimal, as IL-6 concentrations in PBS processed samples from 157 severe asthma patients (median 46.52 pg/ml) were found to be significantly higher (p<0.0001) than in DTT processed samples (median 13.3 pg/ml). Those with sputum IL-6 concentrations above the median had significantly lower FEV_1 % predicted pre bronchodilator (p=0.022) and post bronchodilator (p=0.012) values than those with sub-median values, although thee was no difference in % reversibility.

Conclusions: These findings of higher sputum IL-6 concentrations in severe asthma and their link to impaired lung function suggest that directed pharmacotherapy against IL-6 may offer benefit in this treatment-resistant asthma group and that sputum IL-6 could be used for stratified selection.

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First in man immunotherapy study with the novel grass pollen allergy vaccine BM32 using the Vienna Challenge Chamber

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Background: Grass pollen are the most important cause of allergic rhinoconjuntivitis in Europe and North America, affecting more that 50% of all allergy sufferers.

Objective: This study is the first in man trial using subcutaneous application of BM32, a novel recombinant grass pollen allergy vaccine.

Methods: The vaccine contains 4 different recombinant fusion proteins constructed from B-cell reactive epitopes derived from the major *Phleum pratense* allergens Phl p 1, Phl p 2, Phl p 5 and Phl p 6, and the PreS protein derived from human hepatitis B virus. 70 patients were randomized to receive 3 subcutaneous injections containing 10, 20 or 40 µg of each antigen adsorbed on alumin or placebo over a period of 8 weeks outside the pollen season. Clinical efficacy was monitored primarily using the difference of mean total nasal symptom score (TNSS) before and after treatment in the Vienna Challenge Chamber. Change in skin reactivity was assessed by titrated skin prick testing. Immunological response was measured by recording changes in allergen specific immunoglobulins (IgE, IgG and IgG isotypes). An analysis of local and systemic adverse events was performed.

Results: Patients in the 20 and 40 µg dose groups showed a significant reduction of TNSS compared to baseline. The threshold of skin reactivity to grass pollen extract was significantly increased in the 40 µg dose group, with a trend towards increase in lower doses. BM32 elicited robust allergen specific increases in total IgG, IgG_1 and IgG_4. Local injection-site reactions occured in almost all patients including placebo. Few patients exhibited systemic reactions to treatment, which in most cases was mild to moderate (grade 0 or I). No serious adverse events were recorded.

Conclusion: Three s.c. injections without updosing of the novel vaccine BM32 resulted in significant improvement of symptoms in the Vienna Challenge Chamber as well as skin reactivity to grass pollen over baseline, due to robust induction of a protective IgG antibody response. The therapy was safe and well tolerated.

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Skin test evaluation of a new vaccine against grass pollen allergy based on recombinant fusion proteins consisting of hepatitis B PreS and peptides from four major grass pollen allergens

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Background: Grass pollen is among the most important elicitors of IgE-mediated allergy. Specific immunotherapy, the only treatment which is able to change the course of allergic disease, is not effective in all patients and may cause side effects.

Objective: Our aim was to investigate the ability of a new vaccine against grass pollen allergy to induce IgE-mediated and/or T cell-mediated reactions by skin prick and atopy patch testing. The vaccine consists of non-allergenic peptides from four different grass pollen allergens (Phl p 1, Phl p 2, Phl p 5, Phl p 6) fused to hepatitis B-derived PreS.

Methods: Sixty grass pollen allergic patients were skin prick tested with 3 concentrations (11µg/ml, 33µg/ml, 100µg/ml) of the four molecules during the grass pollen season. All skin prick tests were performed in duplicates. All patients also underwent atopy patch testing with 80µg/ml and 160µg/ml of a mixture of the four molecules. Commercial grass pollen extract was used as a positive control for both tests.

Results: One patient developed a double-positive reaction to the mix of the molecules at 100µg/ml. No double-positive reactions were seen at 11µg/ml and 33µg/ml. Small, single-positive skin reactions to were observed in 9 cases. No late-phase reactions to the carrier-bound peptides were observed after skin prick testing.

In the atopy patch test, 6/60 patients developed a positive reaction to grass pollen extract while none of the patients reacted with the carrier-bound peptides.

Conclusion: The recombinant PreS fusion proteins did not induce any relevant IgE-mediated reactions in skin prick tests even during the pollen season. Results from atopy patch testing indicate that their ability to induce T cell-mediated side effects is also reduced. These molecules have lower allergenic activity than previously tested recombinant hypoallergens and therefore are promising new vaccine candidates for grass pollen allergy.

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Efficacy and safety of oral immunotherapy for food-induced anaphylaxis

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Background: Oral Immunotherapy (OIT) is current hot topics in food allergy in recent several years. OIT is not yet applied to countermeasure for food-induced anaphylaxis worldwide.

Methods: We introduced very short rush protocol (5 days' admission) followed by slow and maintenance phase follow up protocol for food-induced anaphylactic patients at Sagamihara National Hospital since 2008. By the end of February in 2012, total 196 patients (Hen's egg: 63, Cow's milk: 91, Wheat: 26, Peanuts: 16) were recruited to the therapeutic study. Ethical committee approved our protocol and informed consent was obtained from each patient's guardian. Prior to the therapy, all patients were confirmed to have anaphylaxis by blinded-oral food challenge tests to causative foods. Target intake protein content of causative foods by the therapy was 6.2 g for heated-hen's egg (HHE), 6.8g of raw cow's milk (RCM), 5.2g of wheat noodle (W), 0.8g of peanuts (P), respectively.

Results: Sixteen patients had dropped out from the protocol either in rush phase (n=5) or during follow-up phase (n=11), mostly due to intake difficulty. During rush phase, moderate to

severe adverse reactions were observed by 17.6% (HHE), 18.2% (CCM), 9.9% (W), and 2.9% (P), respectively. During follow-up phase, moderate to severe adverse reactions were observed by 1.5% (HHE), 1.7% (CCM), 1.5% (W), and 0% (P). Regarding efficacy during rush phase, reaching to target intake protein content of causative foods was achieved by 78% (HHE), 72.2% (RCM), 87.5% (W), and 60.0% (P). At 1 year after rush phase, desensitization with target intake protein content was achieved by 95.8% (HHE), 64.1% (RCM), 100% (W), and 100% (P). However, tolerance acquisition (confirmed by 2 weeks' complete elimination and subsequent oral food challenge test) was only by 37.5% (HHE), 10.3% (RCM), 72.2% (W), and 50.0% (P).During follow-up phase, antigen-specific IgE continued to decline with some elevation after rush phase, whereas antigen specific IgG4 elevated.

Conclusions: Our protocol for food-induced anaphylactic patients seems to be effective regarding desensitizing severe food anaphylactic patients, however, tolerance acquisition for those severe food anaphylactic patients seems to be far behind the desensitization.

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Diamine oxidase: A novel biomarker for monitoring ex-vivo allergen-induced basophil reactivity following grass pollen immunotherapy

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Background: Although the suppression of the early allergic response following *in-vivo* cutaneous grass pollen challenge is associated with decreased basophil histamine release, the effects of subcutaneous (SCIT) and sublingual immunotherapy (SLIT) on *ex-vivo* grass pollen-induced basophil reactivity is yet to be fully determined. We hypothesized that grass pollen SCIT and SLIT are associated with reduced *ex-vivo* allergen-induced basophil reactivity as measured by basophil surface activation markers (CD203c, CD63 and CD107a) and intracellular diamine oxidase incorporation as detected by flow cytometry, a novel assay of basophil histamine release. We further hypothesized that reduced basophil reactivity persist following withdrawal SLIT.

Method: In a cross-sectional study, we assessed *ex-vivo* grass pollen-induced basophil reactivity in patients who underwent SCIT (n=7), SLIT (n=7), SLIT withdrawal (SLIT-TOL) (n=4) and untreated grass pollen allergics (n=10). $CD203c^{bright}$, $CD63^{+}$, $CD107a^{+}$ and intracellular fluorochrome-labelled diamine oxidase $(DAO)^{+}$ $CRTh2^{+}$ basophils were measured by whole blood flow cytometric assay. Immunereactive sIgG4 and functional sIgG4 antibodies were measured by ImmunoCAP system and IgE-facilitated allergen binding assay, respectively.

Results: Rhinitis total symptom scores (RTSS) were significantly reduced in SCIT (p=0.01), SLIT (p=0.004) and SLIT-TOL (p=0.01) groups compared to allergics. SCIT- and SLIT-treated groups had significantly decreased proportion of $CD203c^{bright}$ (p=0.002; p=0.001), $CD63^{+}$ (p=0.002; p=0.001) and $CD107a^{+}$ $CRTh2^{+}$ basophils (p=0.04; p=0.001) compared to allergics. $DAO^{+}CRTh2^{+}$ basophils were not decreased following SCIT (p=0.0007) or SLIT (p=0.0004) compared to allergics. Interestingly, *ex-vivo* grass pollen-induced basophil hyporeactivity persisted despite treatment withdrawal in SLIT-TOL group ($CD203c^{bright}$ (p=0.005), $CD63^{+}$ (p=0.006) and $CD107a^{+}$ $CRTh2^{+}$ basophils (p=0.008). DAO^{+} $CRTh2^{+}$ basophils did not significantly decrease following *ex-vivo* allergen stimulation when compared to allergics (p=0.01). SCIT, SCIT and SLIT-TOL groups had increased concentrations of grass pollen-specific IgG4 antibodies compared to untreated allergic controls (p<0.001; p=0.008;p=0.005). A significant correlation was revealed between RTSS and proportion of $CD203c^{bright}$, $CD63^{+}$ and $DAO^{+}CRTh2^{+}$ basophils measured in all groups (spearman rank; r=0.57, p=0.003; r=0.52, p=0.0008; r=-0.73; p<0.0001) respectively.

Conclusions: Our findings suggest that assessments of surface CD203c, CD63, CD107a and intracellular labeled-DAO on $CRTh2^{+}$ basophils may be useful predictive biomarkers of the clinical response to immunotherapy. This needs to be evaluated in a large randomized double-blind placebo controlled study.

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Two year follow up after rush oral immunotherapy in hen's egg-induced anaphylactic children

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Background: The objectives of this study are to investigate the efficacy and safety of egg-OIT to induce the desensitization and tolerance in egg-induced anaphylactic children.

Methods: Forty two anaphylactic subjects (average age: 8.8±2.2, male-female ratio:1.2) underwent egg-OIT consisted of 3 phases: rush, build-up, and maintenance. In all phases, heated egg was used in all subjects. Among 42 subjects, 38subjects (6-16 years old), who had received rush-OIT in admission and completed the build-up/maintenance phase at least 12 months in our outpatient, were analyzed. The goal of the build-up OIT was the ingestion 60g of heated-egg daily. After reaching this level, subjects had taken this dose until the asymptomatic duration for more than 3 months (maintenance-OIT).The subjects fulfilled this criteria underwent the oral food challenge (OFC) to confirm the tolerance acquisition after the cessation of egg ingestion for 2 weeks.

Results: The threshold to induce anaphylaxis was 9.1g as heated-he's egg, and mean specific IgE to egg white was 24.4±22.7Ua/ml. After the two year OIT, 28 subjects (93%) among 30 could have ingested 60g of egg asymptomatically and underwent the final OFC. Thirteen subjects (43%) passed the OFC confirming oral tolerance acquisition. Compared to the onset, egg-white and OVM specific IgE significantly decreased (24.4±3.6 vs 11.4±2.7, 21.4±3.4 vs 7.8±1.4) at the time of OFC, whereas there was not statistically significant difference in these IgE titers between subjects with or without tolerance. The frequency of immediate reactions induced by OIT in subjects with tolerance was significantly lower than that in those without tolerance (0.08±0.07 vs 1.4±0.4,%, p<0.01).

Conclusions: This study suggests that egg-OIT could induce clinical desensitization effectively for egg anaphylactic children,

but not all of them progressed toward clinical tolerance within2 years. To induce clinical tolerance, maintenance-OIT for some patients should continue for a few years. Compared with the milk-OIT, we hardly experienced the adverse effects during egg-OIT. Among 42 egg-OIT, we have experienced a case developing eosinophilic colitis as an adverse event. Further and longer study to clarify the efficacy and the safety of egg-OIT for the most severe cases are needed.

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Enhancement of the frequency and function of IL-10-secreting type I T regulatory cells after one year of cluster allergen-specific immunotherapy

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Background: Allergen specific immunotherapy (SIT) is a highly effective treatment for allergic diseases, but the underlying mechanisms are unclear. In this study, we investigated the effects of cluster SIT with *Dermatophagoides pteronyssinus (Der p)* on IL-10-secreting type I T regulatory (Tr1) cells in *Der p*-sensitized patients with allergic rhinitis.

Methods: This was a prospective randomized study involving 68 participants (aged 18-60 years); of whom 38 were patients with allergic rhinitis (AR) and received *Der p*-SIT for one year and 30 were non-allergic controls. $IL\text{-}10^{+}IL\text{-}4^{-}$ $CD4^{+}$ T cells were measured by flow cytometry for the patient group at baseline and at the end of one year of SIT, and for non-allergic controls. Similarly, IL-10 in supernatants from allergen-stimulated peripheral blood mononuclear cells (PBMC) cultures was measured by ELISA, and the suppressive effect of Tr1 cells on cell proliferation and cytokine release (IFN-γ and IL-4) in PBMCs was estimated in cultures from both groups. Allergen-specific serum IgE and IgG4 were also assessed by RAST and ELISA for the SIT group.

Results: The levels of IL-10-secreting Tr1 cells, IgG4 and allergen-induced IL-10 synthesis from PBMC cultures were significantly increased and the function of Tr1 cells was enhanced after one year of SIT compared to baseline levels. In contrast, the level of IgE was not significantly changed.

Conclusion: These data suggest that the cluster *Der p*-SIT may enhance the frequency and function of IL-10-secreting Tr1 cells.

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Poster Abstracts Session 11

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Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation

Muhammad Asaduzzaman, Courtney Davidson, Narcy Arizmendi, John Robert Gordon, Morley D. Hollenberg, Harissios Vliagoftis

Introduction: Serine proteinases are increased in the airways of asthmatic individuals and may participate in the development of airway inflammation. A number of serine proteinases released by inflammatory cells can activate protease-activated receptor 2 (PAR_2). We have previously shown that PAR_2 activation in the airways leads to airway hyperresponsiveness (AHR) and inflammation, thus implicating PAR_2 in asthma pathogenesis. We now hypothesize that functional inhibition of PAR_2 during allergen challenge inhibits allergen-induced AHR and airway inflammation in murine models of asthma.

Methods: Balb/c mice were sensitized intraperitoneally with ovalbumin (OVA) in the presence od aluminum hydroxide followed intranasal (i.n.) challenge with OVA. To investigate the role of PAR_2 in the development of AHR and airway inflammation we administered a blocking anti-PAR_2 monoclonal antibody (SAM-11) or an isotype matched control antibody i.n. before every allergen challenge (prevention model). In a separate set of experiments we administered the anti- PAR_2 antibody after inflammation was already established (treatment model). AHR and airway inflammation were assessed 24h after the last allergen challenge in both models.

Results: Administration of the anti-PAR_2 antibody, but not the isotype matched control antibody, in the prevention model, significantly inhibited OVA-induced AHR, accumulation of inflammatory cells in the airways and the release of inflammatory mediators such as eotaxin in the lung tissue. Treatment of animals *in vivo* with the anti- PAR_2 antibody in the prevention model also decreased ex vivo antigen-specific proliferation of spleen cells, but had no effect on allergen-specific serum immunoglobulin levels. Similarly, administration of the anti PAR_2 antibody in the treatment model inhibited AHR and all measurements of airway inflammation.

Conclusions: Our findings suggest that administration of an anti-PAR_2 antibody during allergen challenge decreases allergen-induced AHR and airway inflammation in mice. Therefore, topical PAR_2 blockade in the airways may be a viable therapeutic approach in allergic asthma.

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Metformin attenuates lung inflammation and remodeling via activation of adenosine monophosphate-activated protein kinase

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Background: Airway remodeling is characterized by structural changes of airway constitutional cells accompanied by increased subepithelial fibrosis and airway smooth muscle (ASM) mass.

Metformin was reported to modulate fibrogenic response in hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase (AMPK) but its role in airway remodeling remains unclear. Here, we report that metformin decreases airway inflammation and remodeling in murine chronic asthma model and inhibits transforming growth factor beta (TGFbeta)-induced fibrogenic property of airway fibroblasts.

Method: We used AMPK-alpha1 heterozygous mice and made chronic asthma model to find role of metformin in chronic airway inflammation and remodeling.

Results: Metformin significantly attenuated fungal protease induced chronic airway inflammation, as shown by attenuation of the influx of total inflammatory cells in bronchioalveolar lavage fluid and production of IgE and IgG1. Expression of alpha-smooth muscle actin and growth factors related to airway remodeling such as TGFbeta1 and VEGF were significantly decreased in metformin treated mice lung. Since fibroblast is the main cell related to airway remodeling, we tested the antifibrogenic role of metformin in human airway fibroblast cell line, IMR-90. Metformin significantly reduced production of fibronectin in response to TGFbeta1 treatment via activation of AMPK in IMR-90 cells. Interestingly, metformin treatment reduced the expression of Smad3, which is the main molecule in TGFbeta signaling pathway.

Conclusions: These data suggest that metformin may mitigate airway remodeling by decreasing production of growth factors as well as attenuating TGFbeta signaling.

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Human umbilical cord blood-derived mesenchymal stem cells suppress allergic airway inflammation in murine asthma model

Cho Sang Heon

Background: Current strategy for asthma treatment is the pharmacotherapy with anti-inflammatory properties, and the use of inhaled corticosteroids is the mainstay. However, conventional pharmacotherapy is not fully effective in asthma control, particularly among severe asthmatics. Therefore, it remains the main challenge to develop a novel therapeutic agent for severe asthma. Recent evidence suggests that mesenchymal stem cell (MSC) therapy could be a potential novel therapeutic agent for refractory inflammatory disorders. We aimed to investigate the anti-inflammatory effects of human umbilical cord blood-derived MSC (huMSC) in experimental murine asthma model

Method: BALB/c mice were sensitized to ovalbumin (OVA) by intraperitoneal injection. huMSC was administered via tail-vein before intranasal OVA challenge. Therapeutic effects were assessed using bronchoalveolar lavage (BAL) inflammatory cell profiles, histopathological changes, and methacholine airway hyper-responsiveness. Anti-inflammatory effects were further evaluated by measuring cytokines and immune regulators in the airways.

Results: huMSC administration significantly reduced airway hyper-responsiveness and lung inflammation, particularly eosinophilic infiltration. The treatment effects accompanied immunologic alterations, including the reduction of Th2 cytokines and the induction of interleukin-10, transforming growth factor-β1, and interferon-γ. OVA-specific IgE levels were decreased by huMSC treatment. Expressions of semaphorin 3A/7A and their receptors (neuropilin-1, plexin A1 and C1) were increased by huMSC treatment.

Conclusions: huMSC treatment effectively reduced OVA-induced allergic airway inflammation partly through a mechanism involving immune regulator induction.

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Improved patients' hand grip strength with pMDI by using three fingers pinch method

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Rationale: Drug treatment for asthma or COPD involves inhalants. Metered dose inhalers(pMDIs) are cheaper than others and world-wide prescribed. Instruction sheet usually shows two fingers pinch method, which use thumb and index finger. Most of elderly patients have difficulties to produce sufficient strength to press canisters. The aim of this study is to evaluate patients can make sufficient grip power by using three fingers pinch method.

Methods: The measurement was performed in a patients group who are using pMDI for more than six months. Hand grip strength was measured by using two or three fingers pinch method with hand dynamometer.

Results: A total of 19 (35.2%) men and 35 (64.8%) women (mean age 57 ± 13 years) were included in the study. Younger patients (< 65 year old) produce more stronger hand grip power than elderly patients(13.8±4.1 lb vs. 10.4±3.8 lb, *p=0.005*, respectively). With three fingers pinch method, elderly patient shows increased grip strength than 2 fingers pinch method(10.4±3.8 lb vs. 16.9±6.2 lb, *p=0.000,* respectively).

Conclusions: Although many patients made sufficient power to press pMDI canister. However, elderly patient could make weak power than younger patients. With three finger pinch method most patients show more increased grip power than two finger pinch method. It is needed to change instruction sheets, in which they show figure and instruction regarding the methods for elderly patients.

Poster Abstracts Session 12

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The Canadian Allergy, Genes and Environment Network: AllerGen NCE

Judah A. Denburg, Dean Befus

AllerGen NCE Inc. is a government funded, trans-disciplinary research network of Canada's leading experts in allergic diseases, asthma and anaphylaxis. AllerGen is based at 23 universities

and works in partnership with multi-sectoral organizations and stakeholders to address unmet bio-medical needs; mobilize knowledge to generate new evidence-based public health policies, regulations and strategies; discover new diagnostic tests and therapies; provide publicly accessible information and educational tools; and, ensure an increase in the number of professionals researching and practicing Allergy and Clinical Immunology. AllerGen's overall aim is to improve the quality of life for allergy and asthma sufferers and their families, and lessen the socio-economic burden of, and reduce the risks associated with, these diseases. AllerGen has completed its first 7 year mandate, and been renewed for a second term, during which it will focus on three major Canadian *Legacy Projects*, already developed and launched:

- A national, multi-disciplinary birth cohort to follow the development of allergies and asthma from pregnancy to childhood and beyond, the *Canadian Healthy Infant Longitudinal Development* (CHILD) *Study,* which to date has recruited almost 4,000 families;
- A multi-centred clinical trials consortium with international outreach, the *Clinical Investigator Collaborative* (CIC), in partnership with both international and Canadian biopharmaceutical and biotechnology companies;
- A multi-centred, trans-disciplinary food allergy research consortium, *The Canadian Food Allergy Strategic Team* (CanFAST).

These three national initiatives will be complemented by *Enabling Platforms* of research that AllerGen has established across Canada and internationally:

- *Gene-Environment Interactions*, working to apply genetic, environmental and epigenetic research innovation, with the twin goals of discovering novel therapies and diagnostics, and implementing novel public health interventions and policies;
- *Biomarkers and Bioinformatics,* using standard operating protocols and animal models to develop an integrated, world-leading systems-biology approach to development and commercialization;
- *Patient, Policy and Public Health Research,* focusing on benefits for patients, and incorporating expertise in the social sciences, to inform public health policy, patient and health professional outreach, and educational disease management tools.

In all of these initiatives, AllerGen will continue to position Canada in a leadership role on the world stage in allergy research and innovation.

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GA²LEN - Global Allergy and Asthma European Network of Excellence

Torsten Zuberbier, Cezmi Ali Akdis, Sergio Bonini, Jean Bousquet, Peter Burney, Karin C. Lodrup Carlsen, Boleslaw Samolinski, Ewa Nizakowska-Mogilnilka

The GA²LEN Network of Excellence, is a consortium of more than 90 leading European institutions, partnered with 2 European-wide associations EAACI (European Academy of Allergy and Clinical Immunology) and EFA (European Federation of Allergy and Airways Diseases Patients Associations), multidisciplinary research centres specializing in allergic diseases and over 500 researchers in 25 countries working on new and continuing research.

GA²LEN, created in 2004 under the EU FP6 programme, continues to prove its sustainability even after EU funding concluded, as it continues to grow and strengthen with new collaborating centres joining the network every year.

GA²LEN enhances quality and relevance of European research, disseminates best practice and knowledge to health professionals, patients and policy makers, integrating its research and communicating findings. GA²LEN implements Europe-wide Quality Management Standards in allergy clinical care and harmonization in diagnostic procedures (GA²LEN Skin Prick Test), revising guidelines in line with GRADE (Grading of Recommendations Assessment, Development and Evaluation) recommendations, organizes "Allergy Schools" throughout Europe in cooperation with EAACI which are open to junior, non-junior scientists and health professionals. GA²LEN organizes specialized Training Programmes for continued education and improvement in higher knowledge levels for health professionals.

Novel allergens are rapidly and constantly emerging in inhalant, food and contact allergies. However, a systematic surveillance of allergy trends, as well as an early alert system for the detection of novel allergens is missing, but would prove highly beneficial for Europe's citizens and its policy makers. Consequently, GA²LEN is active in the European Parliament, European Council and was an active participant throughout the EU Polish Presidency calling for GA²LENs existing infrastructure of more than 100 centres covering the European Union offering an ideal and cost effective platform to fill this gap with the GA²LEN Sentinel Network proposal, should the EU use.

GA²LEN continuously strives towards the ultimate goal of reducing the burden of allergy and asthma for Europe's society and economy. GA²LEN continuously aims at developing better health care and improving the daily quality of life for more than 200 million European citizens with allergic diseases. www.ga2len.net

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CfA-The Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden

Sven-Erik Dahlén

The Centre for Allergy Research is a centre of excellence at Karolinska Institutet (KI) established in 1999 after a competitive national application process, with funding partnership currently including also the Stockholm County Council (The Health Care Provider).

During the first operational period, 2001-2005, CfA initiated a transdisciplinary Graduate School in Allergy Research, where 21 students were recruited from a variety of backgrounds to create the next generation of allergy scientists. During the second operational period, 2006-2011, the networking within KI was further strengthened. Proposals for transdiciplinary collaborations

were peer reviewed by an external international panel of scientists and a total of 21 different projects have, up to now, been supported through CfA. Together, these have so far resulted in over 100 publications, many in high impact journals. The collaborative project grants have been particularly well received by junior group leaders in the process of establishing their careers.

The CfA has provided 37 clinicians with "CfA-months", giving them up to 3 months of research time during a one-year period. CfA months have been described as being "worth their weight in gold" and have made a significant difference to clinicians in research career.

The Competence Centre for Experimental Models (CCEM) was initiated in 2009 as a core facility for physiological investigations of airway inflammation in small animals. The CCEM has been used by about 15 research groups at the KI and has also been commissioned by external parties.

In 2009, CfA received a generous donation from the Sandler Foundation to start the postdoc programme: *The Bernard Osher Initiative for Research on Severe Asthma*. The programme funded seven intertwined postdoctoral projects in various disciplines and has been truly translational.

CfA is currently represented by 36 group leaders from eleven institutions across KI, with research being performed by over 150 postdocs and staff as well as 60 PhD students. In addition to networking within KI, CfA researchers collaborate externally with large number of national as well as international institutes and companies.

The mission of CfA is to strengthen collaborative and cutting-edge interdisciplinary research on the major unmet medical needs in asthma and allergy.

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Christine Kuehne-Center for Allergy Research and Education (CK-CARE): Davos – Munich – Zurich

J. Ring, C. Akdis, H. Behrendt, R. Lauener, G. Schaeppi

Christine Kuehne-Center for Allergy Research and Education (CK-CARE), Davos – Munich – Zurich

The Christine Kuehne-Center for Allergy Research and Education (CK-CARE) was founded by the generous commitment of Klaus Michael and Christine Kuehne in July 2009 with the aim to reduce deficits in diagnosis, treatment and prevention of allergic diseases through fostering research and educational activities. The center is organized in five "research areas" at five different locations in the three cities of Zurich, Munich and Davos with the headquarters of CK-CARE in Davos, Switzerland.

Themes of the five research areas are:
A – Environment, allergens and exposure (H. Behrendt, Center for Allergy and Environment, Munich)

B – Immunoepidemiology of allergic diseases in childhood (R. Lauener, Dept. Pediatrics, Zurich)

C – Innovative diagnostics and therapy (J. Ring, Dept. Dermatology and Allergy, Munich)

D – Mechanisms of severe allergy (C. Akdis, Swiss Institute for Allergy Research, Davos)

E – Therapy and rehabilitation (R. Lauener, High Altitude Clinic, Davos)

Through "bridge projects" partners from other institutions in Germany, Austria, Switzerland and beyond have been recruited who contribute to the scientific work of CK-CARE. Through the instruments of regular meetings and visitor exchange programs both for scientific and clinical work at one of the CK-CARE institutions, CK-CARE also serves as a platform where allergy researchers and clinicians can meet.

The second pillar of CK-CARE activity is education targeted to medical students, residents, but especially to the group of primary care physicians. CK-CARE also has helped to establish the program of "anaphylaxis school" in Germany together with the working group Anaphylaxis, Training and Education (AGATE) and is supporting train-the-trainer seminars both for eczema and anaphylaxis school programs in Germany, Austria and Switzerland. Primary care physicians are approached by small group seminars with practical teaching preferably in allergy diagnostics. A brochure "Basic knowledge in allergy – scientific allergy" has been published and distributed to 55,000 primary care physicians with the option to be also readable for interested lay groups and to be left in the waiting room for the patients.

In July 2011 the first "Global Allergy Forum" (GAF) took place in Davos where 40 opinion leaders from four continents and all fields of allergy and related disciplines came together in order to discuss problems and deficits in the actual situation of allergology under the theme "Allergy: Barriers to cure". After three intensive days a consensus document was developed as "Davos Declaration: Allergy as a global problem"[1]. CK-CARE is working together with national and international societies, but also patient organizations especially in Switzerland. CK-CARE has been founded for a first period of five years, but most likely after external peer-reviewed evaluation will be continued and possibly extended to new partners and sustainable common projects. In the first two and a half years the results of research are remarkable; more than 100 publications with a total impact factor of over 750 have appeared under the name or with the support of CK-CARE. The directors of CK-CARE are confident that in the future the successful work will be continued to achieve the mission "Through research and education to a better prevention and disease management in allergy".

1) Davos Declaration: Allergy as a global problem. J. Ring, C. Akdis, H. Behrendt, R. Lauener, G. Schaeppi et al. Allergy (2012) 67: 141–292.

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European Mast Cell and Basophil Research Network (EMBRN)

Gunnar Nilsson, Francesca Levi-Schaffer, Marcus Maurer, Ulrich Blank, Franco H. Falcone, Julia Trosien, Axel Lorentz, Stephan C. Bischoff

The European Mast Cell and Basophil Research Network (EMBRN) is a non-profit organization for all who are interested in the

biology of mast cells and basophils and their functions in health and disease. EMBRN was raised from a former Marie-Curie EU program and was founded in the summer of 2008 during a mast cell meeting in Stuttgart, Germany. It was formalized in 2009 and registered as a legal organization in 2011. The organization originated from an EU project on "Mast cells and chronic diseases" (MCCID, coordinated by U. Blank and funded by the EU Marie Curie from 2003 until 2008). The major goal for EMBRN is to continue these efforts by gathering scientists and physicians from all over the world interested in mast cells and basophils for collaborative scientific projects and international mast cell and basophil meetings. The activities of the EMBRN comprise basic, translational and clinical research associated with mast cells, basophils and related cells. The aim for EMBRN is to increase the knowledge on mast cells and basophils and their functions in health and diseases. The network organizes and supports collaborative research applications, e.g., EU applications and other multinational applications for funding. The network welcomes industrial members interested in mast cell and basophil research and mast cell/basophil-related diseases; and supports scientific collaboration between industrial partners and research institutes. We also promote the development and distribution of unique tools, protocols, reagents and models for mast cell and basophil research. EMBRN successfully applied for and works in close association with the European COST Action BM1007 "Mast cells and Basophils – Targets for innovative therapies"; and organizes regular meetings (2012 in Berlin, December 26-27; www.mcbm.de). For more information about EMBRN and to apply for membership, please visit our home page at www.embrn.eu.

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COST Action BM1007: Mast cell and Basophils - Targets for innovative therapies

Marcus Maurer[1], Gunnar Nilsson[2], Francesca Levi-Schaffer[3], Ulrich Blank[4], Ilkka Harvima[5], Stephan Bischoff[6], Franco Falcone[7], Frank Siebenhaar[1]

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COST is an intergovernmental framework for European Cooperation in Science and Technology, allowing the coordination of nationally-funded research on a European level. COST has the specific mission and goal to reduce the fragmentation in European research and to open the European Research Area to worldwide cooperation. In support of advanced multidisciplinary research, COST fosters to build and frame the European Research Area (ERA). It anticipates and complements the activities of the EU Framework Programs, constituting a "bridge" towards the scientific communities of emerging countries. It also increases the mobility of researchers across Europe and thereby supports the establishment of scientific excellence.

The main objective of this COST Action is the identification and characterization of novel disease-related cell specific targets that will result in the development of innovative therapeutic strategies for the treatment of chronic inflammatory, autoimmune and allergic diseases by focusing on basic, clinical and translational science in mast cell and basophil research across Europe. The Action creates a multidisciplinary approach to mast cell and basophil research in order to increase understanding and to translate this knowledge into the development of potentially beneficial end points. Mast cells and basophils have long been recognized for their detrimental role in the elicitation of allergic diseases. In recent years, scientific results revealed both cell types also as versatile effector cells that exhibit far more complex functions beyond their role in allergy. Mast cells and basophils have been shown to be critically involved in various innate and adaptive immune responses and, thereby, providing beneficial host protecting immunity. They also contribute to the development and maintenance of several chronic inflammatory and allergic diseases which, even at the present time, lack sufficient treatment options. The diversity of important mast cell and basophil functions places these cell types into promising therapeutic targets. The Action creates a network of European experts to foster a multidisciplinary approach for the identification, characterization, and development of such targets and their translation into novel therapeutic strategies.

Oral Abstracts Session 8

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Critical roles for basophils in allergy and protective immunity as revealed by the basophil-engineered mice

Hajime Karasuyama, Takeshi Wada, Kazushige Obata, Soichiro Yoshikawa

Background: Basophils are the least abundant granulocytes and represent less than 1% of peripheral blood leukocytes. Although they are evolutionarily conserved in many animal species, their functional significance remained uncertain, and they were erroneously considered as a lesser relative or precursor of mast cells. Basophil research was long hampered by their rarity and the lack of useful analytical tools. The discovery of mast cell-deficient mouse strains carrying the c-kit mutations has greatly advanced our understanding of mast cell functions *in vivo*. Therefore, the establishment of model mice that are deficient only in basophils is essential for the promotion of the basophil research. We developed a basophil-depleting mAb (Ba103) that is specific for CD200R3, and other researchers found that an anti-FcεRIa mAb (MAR-1) can also deplete basophils when administered *in vivo*. Although both mAbs became powerful tools to study the roles of basophils, we need to consider the possible off-target effect on mast cells, because both CD200R3 and FcεRIa are expressed by mast cells besides basophils. Moreover, some inflammatory DCs reportedly express FcεRIa.

Method: To overcome the limitation of the antibody-mediated basophil depletion, we have recently developed an engineered mouse strain (*Mcpt8*^{DTR}) in that the human diphtheria toxin (DT) receptor is expressed solely by basophils, and therefore basophils can be selectively ablated by the DT administration at any time. We have established another useful model mice (*Mcpt8*^{GFP}) in that only basophils express GFP, and therefore the behavior of basophils can be monitored in living mice.

Results & Conclusion: The efficacy of the *Mcpt8*^{DTR} system was clearly demonstrated by that the DT-mediated basophil depletion completely abolished the development of IgE-mediated chronic allergic inflammation. Taking advantage of this mouse model, we could show that basophils play a pivotal role in the IgE-dependent, acquired protective immunity against tick infestation. Interestingly, the FcεRI expression on basophils but not mast cells was essential for the manifestation of tick resistance, even though both types of cells contributed to it. We are currently analyzing further roles for basophils in allergy and protective immunity, by using *Mcpt8*^{DTR} and *Mcpt8*^{GFP} mice.

Reference: Karasuyama et al., Nonredundant roles of basophils in immunity. *Annu. Rev. Immunol.* 29: 45-69, 2011.

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Gene expression profiles in human basophils after exposure to immobilized human IgG

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Background: Recent studies revealed that basophils play critical roles in mouse models of late-phase skin reactions, antigen presentation and IgG-induced platelet-activating factor (PAF)-mediated systemic anaphylaxis. However, the precise roles of human basophils in the pathogenesis of human diseases remain unclear. In order to investigate whether human basophils respond to IgG-mediated signaling, the mRNA expression profiles of human basophils after exposure to immobilized human IgG were comprehensively analyzed.

Methods: Human basophils were isolated from the peripheral blood of allergic donors by double Percoll gradient sedimentation and negative-selection using immunomagnetic beads. The purity of the basophils always exceeded 95%. The basophils were cultured in plates that had been precoated with human IgG or HSA in the presence and absence of anti-FcεRI mAB. The cells were harvested after 3 or 18 hours of culture, and the mRNA expression profiles were analyzed using a GeneChip system.

Results: After exposure to immobilized IgG, expression of mRNA for metalloproteinase 1 (MMP1) and XCL1 was markedly upregulated, whereas that for PAF-acetylhydrolase (PLA2G7) was markedly downregulated. mRNA expression for lysophosphatidylcholine acyltransferase (LPCAT) was not altered after exposure to immobilized IgG or activation with anti-FcεRI mAb.

Conclusions: Human basophils are possibly activated by immobilized IgG. The precise roles of basophils in the pathogenesis of human anaphylactic reactions, especially those with PAF release should be further studied.

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IgE responses to galactose-a1,3-galactose; involvement of tick Ixodes ricinus in red meat allergy

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Background: The carbohydrate epitope galactose-α1,3-galactose (a-gal) is abundantly expressed on non-primate mammalian proteins. Patients with IgE antibodies against α-gal have reported severe allergic reactions after consumption of red meat (beef, pork or lamb) and associations between induction of IgE to α-gal and tick bites have been reported. The aim was to investigate whether Swedish patients with suspected meat allergy have IgE antibodies to α-gal and to explore if they have IgE to the tick *I. ricinus* that can be linked to the α-gal epitope and whether these responses can be visualized on sections of *I. ricinus*.

Method: Sera from five Swedish patients reporting anaphylaxis or urticaria following consumption of red meat and a history of several tick bites were analysed for IgE to α-gal, beef and chicken using the ImmunoCAP System (Phadia AB, Uppsala, Sweden). Western blotting and competition-ELISA was used for further analysis of IgE responses to the tick *I. ricinus*. Cryostat-cut sections of *I. ricinus* nymphs were probed with patient sera, a polyclonal mouse antibody raised against α-gal or with an irrelevant polyclonal mouse antibody.

Results: All patients were IgE positive to α-gal and beef by ImmunoCAP. Western blotting demonstrated strong IgE responses to *I. ricinus* in serum from all patients and preincubation with α-gal resulted in a significant reduction of IgE responses to *I. ricinus* in two of the sera. Dose-dependent inhibition of IgE to α-gal was achieved in all patients by preincubation of sera with *I. ricinus* extract in a competition-ELISA. Patient sera IgE positive to α-gal and the anti-α-gal polyclonal antibody gave comparable results in immunohistochemistry, predominantly staining the *I. ricinus* gut. Sections probed with patient sera IgE negative to α-gal and an irrelevant polyclonal antibody resulted in no specific staining.

Conclusions: We have for the first time shown that the tick *I. ricinus* contains the α-gal epitope, which can be localised to the GI tract. In addition, patients with delayed severe reactions to red meat in Sweden have IgE responses to beef and *I. ricinus* that can be linked to the α-gal epitope. These results provide further evidence that tick bites are associated with IgE responses to α-gal and red meat allergy.

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Increased hyperoxidized peroxiredoxins in peripheral blood mononuclear cells of asthma patients and polymorphisms in PRDX6 gene are associated with asthma severity

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Background: Oxidative stress is involved in the pathogenesis of asthma, and peroxiredoxins (PRDX) may be critical in controlling intracellular oxidative stress. The aim of this study was to evaluate expressions of hyperoxidized PRDX in asthmatic individuals as well as association of PRDX gene polymorphism with asthma phenotype.

Methods: The levels of expression of PRDX1, PRDX2, PRDX3, and PRDX6 and their hyperoxidized forms (PRDX-SO3) were measured in PBMCs from asthma patients and control subjects. In addition, cells from these subjects were treated with hydrogen peroxide (H2O2) and their intracellular concentrations of reactive oxygen species (ROS) were measured. To further support the association of PRDX with asthma severity, we have checked PRDX6 gene SNPs and evaluated atopy and AHR in 2,055 Korean children and 449 adult asthmatic patients

Results: The ratios of hyperoxidized to total PRDX (PRDX-SO3/PRDX) in PBMCs were significantly higher in asthma patients than in normal subjects and were correlated with disease severity, with the highest ratio seen in patients with severe asthma. Furthermore, H2O2 treatment of PBMCs, particularly lymphocytes, increased intracellular ROS concentrations with greater and more persistent increases observed in cells from asthmatic than from control subjects. Next, we analyzed PRDX6 polymorphisms. We found 7 informative SNPs by direct sequencing and selected two tagging SNPs for scoring, which were -300A>G and -271C>T. Atopy was associated with a haplotype (AC) of the PRDX6 gene (P = 0.015, OR [95% CI] = 1.26 [1.04–1.52]). The -300C>T SNP was significantly associated with AHR (P = 0.004), asthma development (P = 0.025), and asthma severity (P = 0.018 [recessive model], OR [95% CI] = 0.46 [0.24-0.87]).

Conclusions: Hyperoxidation of PRDX may serve as a biomarker of asthma severity and may predict enhanced susceptibility to oxidative stress load in PBMCs of asthmatics. Furthermore, PRDX6 gene SNPs were associated with increased risk of atopy, AHR, and asthma severity, suggesting that PRDX6 may be a good candidate gene target for future asthma therapies.

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Serum periostin levels are correlated with decline of pulmonary function in asthma patients

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Background: We previously demonstrated that periostin, an extracellular matrix protein, contributes to thickening of airway basement membrane, a cardinal feature of asthma. Serum periostin has recently emerged as a "companion diagnostic" to predict the efficacy of anti-IL-13 antibody (lebrikizumab) for patients with inadequately controlled asthma despite inhaled corticosteroids (ICS) treatment (*N Engl J Med, 365*, 1088, 2011). However, the correlation between serum periostin levels and clinical data in asthma patients on ICS, including development of airflow limitation, a functional consequence of airway remodeling, has not been well characterized.

Methods: We developed an ELISA system to detect periostin using two monoclonal anti-periostin antibodies (SS18A and SS17B). Non-smoking adult asthma patients (n=198) who had been treated with ICS for 4 years or more were enrolled. Serum periostin levels and annual changes in FEV1 (ΔFEV1) at least one year after the initiation of ICS treatment to the timing of enrollment or later were assessed.

Results: Serum periostin levels in the overall asthma patients were significantly higher than those of control donors (n=66, 95.5ng/ml ± 38.5 ng/ml vs. 39.2 ng/ml ± 24.5 ng/ml). We divided the patients into two groups, i.e. rapid decliners (RD, ΔFEV1 < -30 ml/yr) and non-rapid decliners (NRD, ΔFEV1 ≥ -30 ml/yr). RD group (n=43) showed higher serum periostin levels than NRD group (n=155, 112.4 ± 49.3 ng/ml vs. 91.2 ± 34.0 ng/ml, p=0.003). More patients in RD group had the severest disease, a history of admission due to asthma exacerbation, complaint of sputum production, and a history of cardiac diseases than those of NRD group.

Conclusions: Serum periostin is a useful biomarker for management of asthma to reflect rapid decline of pulmonary function in the patients on the prolonged treatment with ICS.

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Recombinant fusion proteins consisting of hepatitis PreS-fused non-allergenic Bet v 1 peptides focus blocking IgG towards IgE epitopes and shift T cell responses to a tolerogenic phenotype

Katharina Marth, Isabella Breyer, Margarete Focke, Katharina Blatt, Mohamed H. Shamji, Janice Layhadi, Domen Zafred, Walter Keller

Background: The major birch pollen allergen Bet v 1 has been identified as the culprit allergen in birch pollen and associated pollen and food allergies.

Methods: Here we report the construction of a vaccine for birch pollen allergy which consists of two non-allergenic peptides derived from the major IgE-binding area on Bet v 1. Four recombinant fusion proteins consisting of these peptides and the hepatitis B surface protein, PreS were expressed in *Escherichia coli* and purified to homogeneity.

Results: The fusion proteins lacked IgE reactivity and allergenic activity as tested with sera and basophils from birch pollen allergic patients. The peptides did not induce any relevant proliferation in cultured PBMC from birch pollen allergic patients and thus seemed to lack Bet v 1-specific T cell epitopes whereas the fusion

proteins induced the secretion of the tolerogenic cytokine IL-10. Immunization of rabbits with the fusion proteins induced IgG antibodies which reacted with Bet v 1 and associated pollen and food allergens. Furthermore, they inhibited the binding of allergic patients IgE to Bet v 1 better than IgG antibodies induced with the complete Bet v 1 allergen. The induced IgG antibodies also inhibited Bet v 1-induced basophil activation and CD23-facilitated allergen presentation to B cells.

Conclusion: Our results indicate that a vaccine based on the PreS fusion proteins has the potential to induce protective allergen-specific IgG antibodies *in vivo* and will exhibit low IgE- and T cell mediated side effects. This vaccine should be suitable for the therapeutic and eventually prophylactic vaccination against birch pollen and associated allergies.

Acknowledgment: This work has been supported by an EAACI Research Fellowship, by grants F4605, F4611 of the Austrian Science Fund, by a research grant from Biomay, Vienna Austria and by the Christian Doppler Research Association.

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Fel d 1 peptide immunotherapy for cat allergy is associated with IL-10 production to multiple cat allergens

Mark Larche, Jingyu Mu, Lesley Wiltshire, Cheryl Kipling, Wendy-Anne Smith, Belinda J. Hales, Wayne R. Thomas, Roderick P. Hafner

Background: Allergen-specific immunotherapy is clinically effective and associated with increased IL-10 production, changes in the Th1:Th2 ratio and induction of allergen-specific IgG. Peptide immunotherapy with T cell epitopes from Fel d 1 is also clinically effective, but the immunological mechanisms responsible for protection against challenge with multiple cat allergens are unclear.

Methods: Mechanisms of peptide immunotherapy were investigated by culturing peripheral blood mononuclear cells (PBMC) with cat allergens (rFel d 1, rFel d 4 and rFel d 7), or unrelated recall antigens (*Candida albicans* extract) and determining cytokine secretion (IL-10, IL-4, IL-5, IL-13 and IFNγ) and proliferative responses (^{3}H-thymidine incorporation). Additionally, the frequency of $CD4^+$ T cells synthesizing cytokines (IL-10, IL-4, IL-5, IL-13, IL-17 and IFNγ), and of $CD4^+$ Fel d 1 MHC class II tetramer$^+$ T cells, before and after immunotherapy with a Fel d 1 peptide vaccine, was determined.

Results: Peptide immunotherapy was associated with increased levels of IL-10 in the supernatant of PBMC cultured with cat allergens, but not the recall antigen *C. albicans*. Despite the absence of T cell epitopes from Fel d 4 and Fel d 7 in the peptide vaccine, treatment was associated with significantly higher levels of IL-10 in response to Fel d 4 ($p=0.015$) and Fel d 7 ($p=0.005$). No differences in proliferative responses were detected. No consistent changes in levels of the other cytokines evaluated were observed. The frequencies of cytokine secreting $CD4^+$ T cells and Fel d 1 MHC class II tetramer$^+$ T cells did not change after treatment.

Conclusions: Treatment of cat allergic subjects with a peptide vaccine containing T cell epitopes from Fel d 1 resulted in increased production of IL-10 in response to culture of PBMC with Fel d 4 and Fel d 7, but not to antigens from *Candida albicans*. Thus treatment with a limited number of T cell epitopes from a major allergen may modulate responses to other allergens.

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Design and characterisation of a T cell epitope-based peptide immunotherapy for peanut allergy

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This study aimed to develop a T cell-targeted peptide immunotherapy as a safe treatment option for peanut-allergic individuals.

Background: Peanut allergy is a life-threatening condition for affected individuals world-wide. There is no cure. While whole allergen extracts are routinely used in specific immunotherapy for other allergies, even minute doses can cause severe side effects and even fatalities in treatment of peanut allergy. Proof-of-concept studies in cat allergy demonstrate that short, T cell epitope-based peptides targeting allergen-specific CD4+ T cells without adverse IgE-mediated reactions are effective for allergen-specific immunotherapy.

Methods: T cell lines specific for the major peanut allergens Ara h 1 or Ara h 2 were generated from peanut-allergic subjects using CFSE-based methodology. Specificity of each line was determined to 20mer peptides spanning the respective allergen sequence using proliferation assays. 20mers containing dominant epitopes were selected based on responder frequencies, prevalence of specific T cells and magnitudes of T cell responses. Core T cell epitopes were mapped within selected 20mers using truncated peptide sets and their HLA restriction determined using blocking antibodies and HLA-typing. Peptides encompassing these epitopes were designed and assessed for solubility, stability, IgE-binding, basophil activation and ability to induce T cell proliferation in peripheral blood from peanut-allergic subjects.

Results: Over 200 T cell lines were generated from 25 HLA-diverse peanut-allergic subjects. Nine dominant CD4+ T cell epitopes were identified in Ara h 1 and five in Ara h 2. These epitopes showed HLA-binding degeneracy and were collectively presented on a combination of HLA-DR, -DQ and -DP molecules. Adjacent epitopes were combined into three Ara h 2 peptides and six Ara h 1 peptides, each no longer than 20 residues. Cysteine residues were replaced with structurally conserved but less chemically reactive Serines. The resultant peptides were water soluble and stable, retained T cell reactivity, did not bind IgE or activate basophils and could directly target peripheral blood T cells in peanut-allergic subjects.

Conclusion: This study identifies nine cysteine-free peptides encompassing fourteen dominant T cell epitopes of Ara h 1 and 2 which provide novel and suitable candidates for a therapeutic for peanut-allergic individuals.

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Suppression of allergen-induced cutaneous late responses by repeated low dose intradermal allergen injection

Stephen J. Till, Giusseppina Rotiroti, Mohamed H. Shamji, Stephen R. Durham

Background: Subcutaneous immunotherapy is effective and suppresses allergen-induced cutaneous late responses, with lesser effects on early responses. Injections typically contain 10-20 micrograms of major allergen (WHO 1998). In contrast, subcutaneous immunotherapy with low dose allergen is ineffective (Van Metre et al., 1980). An early anecdotal report (Philips 1926) described low dose allergen inoculation directly into the dermis, an immunologically active area containing abundant dendritic cells and lymphatics. We previously serendipitously noted that repeated nanogram doses of intradermal allergen used for testing tended themselves to be associated with blunting of late skin responses (Francis et al., 2008). The objective of this study was to test the robustness and allergen specificity of this phenomenon.

Method: Thirty adults with dual sensitivity to grass and tree pollens were randomised to receive either i) six repeat intradermal injections at two week intervals of grass pollen extract (7 ng of Phl p 5 per injection) or ii) two intradermal injections separated by 10 weeks, or iii) a single intradermal injection at 10 weeks. Cutaneous early and late responses were then measured following double-blind injection intradermal grass and birch pollen extracts.

Results: Six fortnightly intradermal grass pollen injections were associated with a mean 93% suppression in cutaneous late response size to grass pollen compared to baseline ($P<0.0001$ ANOVA), and mean 29% suppression in early responses ($P<0.001$). In the group that had received six grass pollen injections, sizes of late responses to grass but not birch pollen, were smaller than in the control groups who had received either two injections separated by 10 weeks ($p<0.01$; Student's t-test)) or a single injection ($p<0.001$). Increases in allergen-specific IgG were observed only in the six injection group and this was associated with increased functional serum inhibitory activity against IgE-allergen complex binding to B cells (IgE-FAB).

Conclusions: Low dose intradermal allergen injections, like conventional subcutaneous high dose immmunotherapy, suppresses allergen-induced late skin responses in a manner that is allergen-specific and associated with induction of inhibitory IgG antibodies. These data form the basis for a phase II randomized controlled efficacy study of low dose intradermal allergen as prophylaxis aganbist natural pollen exposure.

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Targeting IgE: A novel therapeutic option in severe atopic eczema

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Background: Patients with severe manifestations of atopic eczema (AE) commonly have grossly elevated serum IgE-levels. Until today, the pathogenetic role of IgE in AE remains a controversial issue. To evaluate the role of IgE in AE, we developed strategies to target IgE in this particular patient population. In a first approach, we conducted a pilot study combining extracorporal immunoadsorption (IA) followed by anti-IgE-antibody omalizumab (OMZ) for maximum possible IgE serum level reduction in patients with severe, therapy-refractory AE and elevated IgE levels. To this end, an investigator-initiated trial (IIT) was performed. The aim of the IIT study was to design, execute and evaluate a therapeutic method based on the initial reduction of elevated serum IgE levels by IA followed by a sustained anti-IgE therapy with OMZ in patients with severe AE.

Methods: Investigator-initiated open-label pilot trial, treating 10 phenotyped and genotyped patients with a 2-4 day cycle of IA using a global extracorporal immunoglobulin adsorption system that also targets IgE, prior to regular subcutaneous administration of 450 mg OMZ every 2 weeks for 6 months, followed by a 6-month observational follow-up. On every visit, total and free IgE as well as TARC (CCL17) levels were quantified in serum. The severity of AE was documented by standardized photos as well as rated by SCORAD and by the patients' personal evaluation on a severity and pruritus-scale.

Results: Before starting treatment, IgE-levels ranged from 3,728 kU/L to 69,872 kU/L. IgE-levels were seen to be reduced significantly after the IA and to continuously drop in all patients during the anti-IgE-therapy (reaching free IgE levels <150 kU/L in 5/10 and <1,000 kU/L in 9/10 patients). A reverse trend was observed during the follow-up period. Parallel, a clear improvement of AE was seen during the treatment period in all patients followed by an aggravation during observational follow-up (SCORAD, pruritus, severity scale, TARC). Two patients dropped out after initial improvement due to acute exacerbation despite anti-IgE therapy with OMZ and one patient was lost due to lack of compliance.

Conclusions: The sequential combination of an IgE targeting therapeutic approach consisting of IA followed by OMZ is suitable to effectively reduce grossly elevated serum IgE levels and to improve clinical symptoms of severe refractory AE without other systemic treatments. Due to the limited number of patients

included and the open-label design in our pilot trial, further studies are needed to strengthen this conclusion based on our novel and promising therapeutic approach. We will address this further with approaches currently under investigation and/or development: i) the use of an IgE-only adsorption system with significantly higher IgE binding capacity, and ii) the design of new and appropriately controlled clinical studies based on the wealth of information from this pilot clinical trial.

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A neuro-endocrine anti-inflammatory pathway: Translation from rats to humans

Dean Befus, Christopher St. Laurent, Katherine Morris St. Laurent, Ron Mathison, Joseph S. Davison

Introduction: The rat protein submandibular gland rat 1 (SMR1) is a pro-hormone regulated by the autonomic nervous system. Various peptides derived from the prohormone play roles in analgesia, erectile function and regulation of inflammation. One anti-inflammatory peptide TDIFEGG, and its derivative FEG and associated mimetics, have anti-inflammatory activity in preclinical models of asthma, food allergy, acute pancreatitis, spinal cord injury, and septic shock. Phase 1 studies in humans have been completed and Phase 2a studies in allergic asthma are scheduled for 2012.

As *Smr1* is not present in the human genome, we analyzed the relevant chromosomal region in humans for genes and their proteins with a similar peptide sequence to TDIFEGG in SMR1. We have identified CABS1 (calcium-binding protein, spermatid-specific 1) as a protein with a similar, putative anti-inflammatory peptide sequence (TDIFELL) and have begun to identify and characterize CABS1 expression in human tissues, and test human peptide sequences for anti-inflammatory and anti-anaphylactic activity.

Methods: We produced rabbit polyclonal antibodies to peptides derived from CABS1 and used 1-D and 2-D western blots, as well as PCR to identify and characterize expression of CABS1 in human salivary glands. An ex-vivo model of allergen (ovalbumin)-induced rat ileal contraction was used to test anti-inflammatory activity of three human peptide sequences.

Results: CABS1 protein and mRNA were found in human salivary glands by western blot and PCR. In experiments involving positive and negative control peptides, three human peptide sequences (10^{-6} to 10^{-8} M) reduced (up to 40%) antigen-induced ileal contractions.

Conclusions: We have identified CABS1 as a candidate anti-inflammatory protein, shown that it is expressed in human salivary glands, and that peptides derived from CABS1, as peptides from SMR1, have anti-inflammatory activity in a model of intestinal anaphylaxis. Thus, we have uncovered a human protein, at least partially homologous in sequence and function to that of a neuroendocrine, anti-inflammatory pathway in rats. Future studies will determine if this postulate is correct, elucidate regulation of CABS1, develop biomarkers of its activity and investigate therapeutic implications.

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