Translational Science:
From Basic to Clinical Immunology and Allergy

Final Program

28th Symposium of the
Collegium Internationale Allergologicum

25-30 APRIL 2010
Ischia, Italy

COLLEGIUM INTERNATIONALE ALLERGOLOGICUM
CIA

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28TH SYMPOSIUM

Collegium Internationale Allergologicum

Translational Science: From Basic to Clinical Immunology and Allergy

ISCHIA, 25-30 APRIL 2010

Dear Colleagues,

It is with great pleasure that we welcome you to the 28th Symposium of the *Collegium Internationale Allergologicum* on ***Translational Science: From Basic to Clinical Immunology and Allergy*** from **25-30 April 2010** in **Ischia, Italy**.

The purpose of the meeting is to bring translational scientists, clinical immunologists and allergists together to foster interdisciplinary approaches to understand and treat immune-based disorders.

As in previous years, the majority of the scientific program will be given over to free communications that will be presented either as oral abstract presentations 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 contains many late-breaking developments.

We believe that the venue we have chosen to accommodate scientists and accompanying persons follows the traditions and style that characterize the meetings of the *Collegium*. Ischia is a truly inspirational island to visit from a historical perspective as well as in regards to social events. Its rich culture reflects its exciting past. Known as the "Green Island" for its abundance of woods and gardens, it was the first site in Italy to be colonized by the Greeks, "Magna Grecia." The Greeks were attracted to the island by the many hot springs, whose healing properties still attract visitors worldwide. The Archeological Museum and Excavations of Pithecusae are also just across the road from the venue.

On behalf of the *Collegium* Council Members, thank you for attending the 28th Symposium in Ischia! 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 Ischia.

Yours sincerely,

Gianni Marone, MD FRCP
President and Symposium Organizer

Massimo Triggiani, MD PhD
Symposium Organizer

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Alain L. de Weck Travel Grant Recipients

For the first 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 grant were awarded to the following attendees:

Moran Elishmereni, Israel
Stefan Feichtner, Austria
Mitchell Grayson, United States
Simon Hogan, United States
Rosetta Pedotti, Italy
Catherine Ptaschinski, Australia
Angela Simpson, United Kingdom
Krisztina Szalai, Hungary
Meri Tulic, Australia
Luca Vangelista, Italy
Michael Wallner, Austria
Hilary Whitworth, United Kingdom

Travel Grant Recipients will be awarded with a certificate presented by Professor Alain L. de Weck during the Gala Dinner on 29 April 2010.

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

CSL Behring, Italy
Indoor Biotechnologies

General Information

The 28th Symposium of the *Collegium* is held on the beautiful island of Ischia, located in the Bay of Naples, 10 km off the western coast of Italy.

Ischia, historically called Pithecusae, has been colonized by the Greeks, Romans, Saracens, Turks, and Aragonese; all of whom were interested in its excellent strategic position as well as its therapeutic hot-spring waters and of course its attractive landscape. The result of all this varied history can be seen in the ruins of various outposts, towers, and "tufa" rock shelters hidden all over the island.

Ischia, the "Green Island," is extraordinary because it offers nature, culture, history and myth within a framework of breathtaking landscape and picturesque architecture.

Airport Transfers

Transfers to and from the Naples Airport or the Naples Central Train Station are included in the registration fee. *Please confirm your departure pick-up time at the Registration Desk located in the Angelo Rizzoli Conference Centre (ARCC).*

CME

The 28th Symposium of the *Collegium Internationale Allergologicum* will offer 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.

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 Angelo Rizzoli Conference Centre (ARCC).

Coffee Breaks

Coffee breaks are included in the registration fee for delegates and will be served daily. Coffee will be served outside the Angelo Rizzoli Conference Centre (ARCC) and in the Gallery of Posters; please check the Schedule of Events for exact times.

Currency

The currency used in Ischia is the Euro. 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 Italy is AC - the cycle is 50Hz 220V. The European plug with round prongs is used.

Evaluations

Evaluation forms are included in the registration packet. Please fill out your form and return it to the Registration Desk in the Angelo Rizzoli Conference Centre (ARCC).

Hospitality Desk

The Hospitality Desk is located in the lobby of the Regina Isabella Hotel. Delegates and accompanying persons will be able to sign up for excursions and ask for advice regarding activities in Ischia.

Hours:

Monday, 26 April	8:00 – 12:00
Wednesday, 28 April	7:00 – 11:00
Thursday, 29 April	7:00 – 11:00

Language

The official language of the 28th Symposium is English, and the official language of Ischia is Italian. English and German are also widely spoken on the island.

Lunches

Buffet-style lunches will be served in the Regina Isabella Restaurant during the following times:

Monday, 26 April	13:30 – 14:45
Wednesday, 28 April	13:15 – 14:30
Thursday, 29 April	13:00 – 14:15

Lunch is included in the registration fee for delegates, accompanying persons and children.

Oral Abstract Sessions

Oral Abstract Sessions will take place on 26, 27, 28, 29 and 30 April. All Oral Abstract Sessions will take place in the Angelo Rizzoli Conference Centre (ARCC), which is directly across the Santa Restituta Square from the headquarter hotel, the Regina Isabella.

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 chairpersons to keep the session on time.

Poster Sessions

Poster Sessions will take place on 26, 28 and 29 April in the Gallery of Posters, which acts as the walk-way to the Angelo Rizzoli Conference Centre (ARCC). 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. *Authors should set posters on Monday, 26 April 2010 before 17:00.*

Proceedings

Papers from the 28th Symposium will be published by Pacini Editore.

Online submission is now open at http://mc.manuscriptcentral.com/cia2010.

Deadline for Submission: 30 June 2010

Length of Manuscript

Oral presentations: 3 printed pages. This is equivalent to approximately 6.0 double spaced manuscript pages, including tables, illustrations and references.
Poster presentations: 2 printed pages. This is equivalent to approximately 4.0 double-spaced manuscript pages, including tables, illustrations and references.

Please note that per figure and table, 0.5 printed page has to be calculated.

General Information

Registration

The Registration Desk is located in the Angelo Rizzoli Conference Centre (ARCC) on all days except Sunday, 25 April, when it is located in the lobby of the Regina Isabella Hotel.

Hours:

Sunday, 25 April	14:00 – 22:00 (at Regina Isabella Hotel)
Monday, 26 April	6:30 – 13:30 15:00 – 19:00
Tuesday, 27 April	7:30 – 12:00
Wednesday, 28 April	7:30 – 13:15 15:00 – 20:00
Thursday, 29 April	7:30 – 13:00 16:00 – 18:45
Friday, 30 April	8:30 – 13:00

Registration Fees

The registration fee for delegates includes:

Airport Transfer on Arrival

- From Naples International Airport or Naples Central Train Station to the port in Naples
- Ticket for either the hydrofoil or ferryboat
- From the port in Ischia to the Hotel

Airport Transfer on Departure

- From the Hotel to the port in Ischia
- Ticket for either the hydrofoil or ferryboat
- From the port in Naples to the airport or train station

Oral Abstract Sessions

Poster Sessions

Coffee Breaks

Lunches

Social Events

The registration fee for accompanying persons and children includes:

Airport Transfer on Arrival

- From Naples International Airport or Naples Central Train Station to the port in Naples
- Ticket for either the hydrofoil or ferryboat
- From the port in Ischia to the Hotel

Airport Transfer on Departure

- From the Hotel to the port in Ischia
- Ticket for either the hydrofoil or ferryboat
- From the port in Naples to the airport or train station

Lunches

Social Events

Speaker Preview Room

The Speaker Preview Room is located in the Angelo Rizzoli Conference Centre (ARCC). Speakers will be able to check and upload their presentations before the Oral Abstract Sessions.

Hours:

Monday, 26 April	6:30 – 17:00
Tuesday, 27 April	7:30 – 12:00
Wednesday, 28 April	7:30 – 17:00
Thursday, 29 April	7:30 – 17:00
Friday, 30 April	8:30 – 13:00

Time Zone

Ischia is on Central European Time (CET), which is one hour ahead of Greenwich Mean Time (GMT).

Tipping

In Italy service is normally included in the restaurant or bar bill, and additionally in most restaurants a cover charge, which ranges from 1 to 3 Euros per person, is automatically added to the check. Tipping cab drivers is unusual, but appreciated, especially if they help you with your luggage. Tipping hotel porters is also appreciated.

Venue

The scientific program of the 28th Symposium will take place in the Angelo Rizzoli Conference Centre (ARCC), which is directly across the Santa Restituta Square from the headquarter hotel, the Regina Isabella. Posters will be displayed in the Gallery of Posters, which acts as the entrance to the Conference Centre.

Social Events

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

Welcome Reception

Sunday, 25 April 2010, 20:00 – 23:00

The Welcome Reception will be held poolside at the Regina Isabella Hotel. The pool is set on the sea shore of Lacco Ameno, on the Bay of Naples. Musical entertainment will be provided along with refreshments and an assortment of hors d'oeuvres.

Boat Ride

Tuesday, 27 April 2010, 12:00 – 18:30

Boats will depart from the Lacco Ameno Pier at 12:00

Following in the tradition of past *Collegium* meetings, a boat ride will take place on the third day of the meeting. Participants will board boats near the Regina Isabella Hotel at the Lacco Ameno Pier at 12:00. Boats will travel around the island from Lacco Ameno to Ischia for lunch at local restaurants at 13:00. The boats will then depart from Ischia at 15:00 with a stop in St. Angelo for shopping and sightseeing. Participants will board the boats in St. Angelo at 17:00 to travel back to Lacco Ameno, arriving at 18:30.

Pizza and Pasta Party

Wednesday, 28 April 2010, 20:15 – 23:00

Buses will depart from the Regina Isabella Hotel at 20:15

A casual Pizza and Pasta Party will take place at the Hotel Continental Terme in Ischia Porto. A variety of Neapolitan foods will be served.

Gala Dinner

Thursday, 29 April 2010, 19:00 – 24:00

An elegant dinner will be held on the last evening of the Symposium at the Regina Isabella Restaurant. The indoor restaurant, with its panoramic windows facing the bay, will provide the perfect setting for the dinner. The hotel restaurants are well known for fine Mediterranean cuisine and a spectacular wine list. Musical entertainment will be provided.

Pompeii

Optional Excursions

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

To register for a tour, please visit the Hospitality Desk, located in the lobby of the Regina Isabella Hotel or the Registration Desk, located in the Angelo Rizzoli Conference Centre (ARCC).

Complete Island Tour

Monday, 26 April 2010
9:00 – 17:30

The Ischia Complete Island Tour will depart from Lacco Ameno and stop in Ischia Ponte, which is the most ancient place in Ischia. Ischia Ponte is a charming seaside settlement dominated by the picturesque Aragonese Castle. Time will be given to tour the Aragonese Castle, the most impressive historical monument in Ischia, which stands on a volcanic rock connected to the island by a bridge built in 1438. Lunch will be provided at a typical restaurant in St. Angelo with a guided tour of the fisherman's village. Lastly, a stop will be made a local Ischia wine cellar for a wine tasting before returning to Lacco Ameno.

Price per person: €70

Cost of tour includes lunch, ticket to Aragonese Castle, English-speaking guide, transportation and wine tasting.

Capri

Wednesday, 28 April 2010
8:00 – 17:00

Famous for its natural beauty, deep-rooted history, mild climate and unparalleled landscape, the island of Capri is a favorite destination. It is located 17 nautical miles south of Naples and 3 miles from the Sorrentine peninsula. In Anacapri, participants will visit the famous Villa San Michele, created by Axel Munthe. From Anacapri, participants will descent to Capri, with its famous piazzettas, bars, cafes and shops. Lunch will be at a typical restaurant and free time will be given for shopping, walking the narrow streets or to visit the beach of Marina Piccola.

Price per person: €80

Cost of tour includes boat ride to and from Capri, English speaking guide and lunch.

Pompeii

Thursday, 29 April 2010
8:00 – 18:00

Participants will depart from Lacco Ameno for transfer by boat to Naples, where a bus will take them to Pompeii. Pompeii has been a popular tourist attraction for nearly 250 years, since its accidental rediscovery in 1748. Along with Herculaneum, its sister city, Pompeii was destroyed and completely buried during a long catastrophic eruption of the volcano Mount Vesuvius spanning two days in AD 79.

The volcano collapsed higher roof-lines and buried Pompeii under 60 feet of ash and pumice, and it was lost for nearly 1,700 years. Since then, its excavation has provided an extraordinarily detailed insight into the life of a city at the height of the Roman Empire. Today, this UNESCO World Heritage Site is one of the most unique attractions in Italy.

Price per person: €85

Cost of tour includes transportation by boat and bus, English speaking guide, lunch and entrance fees.

La Mortella

Thursday, 29 April 2010
9:45 – 13:00

Sir William Walton, one of the greatest English composers of the 20th Century, set up his home on Ischia almost 50 years ago with his Argentinean wife Susana and entertained many famous houseguests over the years. The Walton property, La Mortella, includes a wonderful exotic tropical garden that is renowned throughout Europe. The garden hosts a collection of plants from different parts of the world, such as tree ferns from Australia and New Zealand, proteas and aloes from South Africa, yuccas and agaves from Mexico, as well as magnolias, camellias, bauhinias, palm trees and cycads.

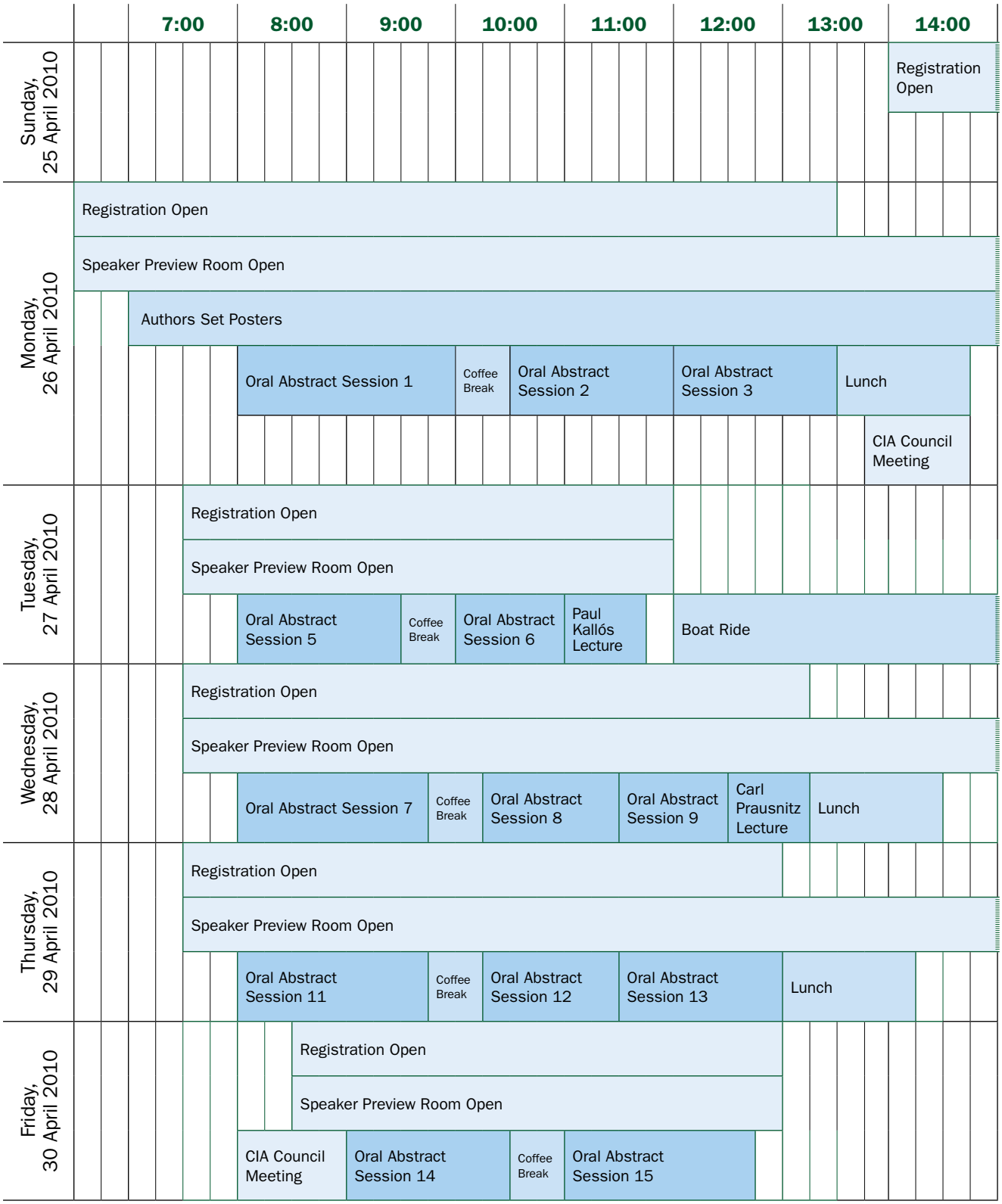
Price per person: €25

Cost of tour includes transportation and entrance fees.

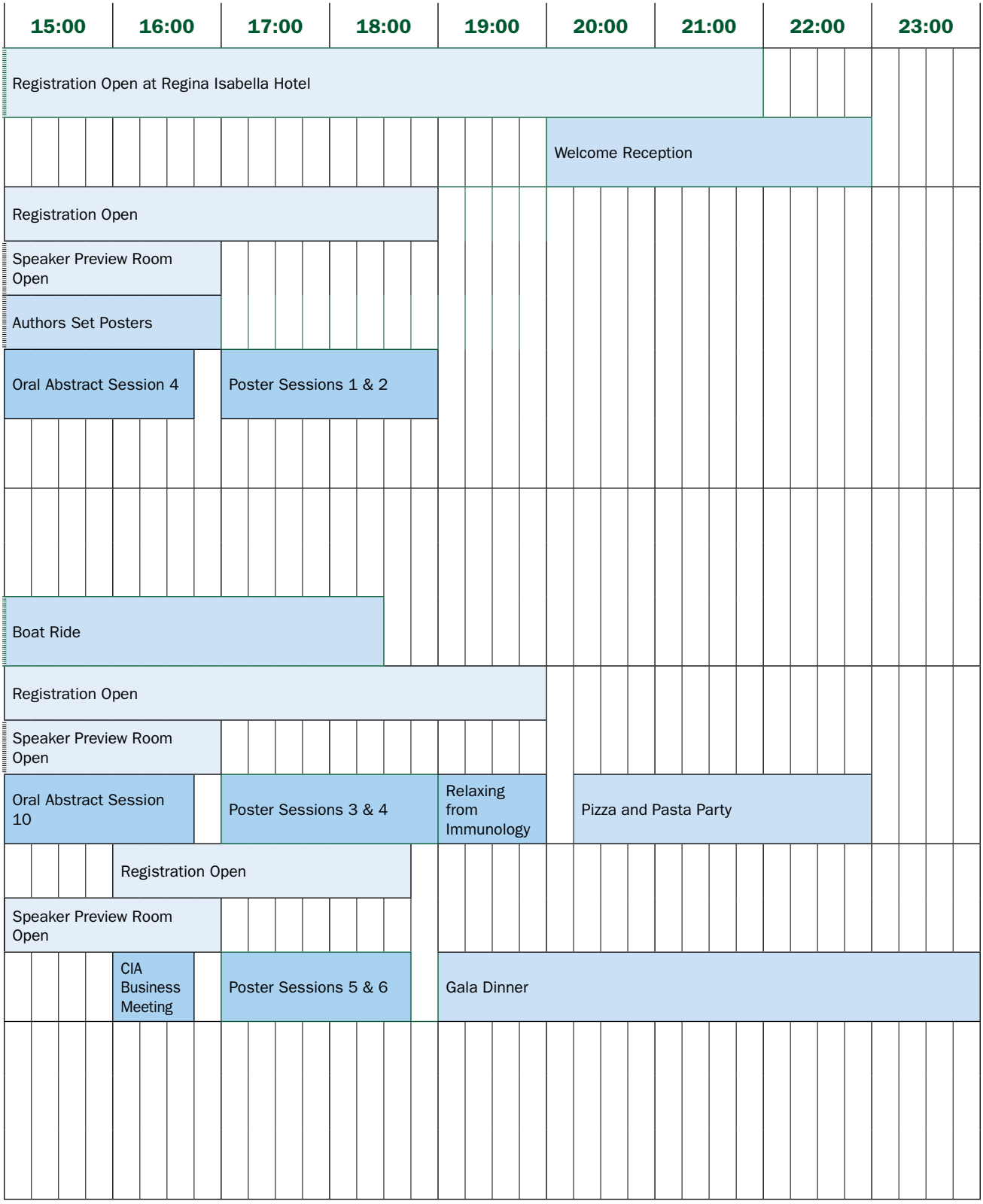
View from Capri

Lacco Ameno Map

Program-at-a-Glance



Program-at-a-Glance



Schedule of Events

Sunday, 25 April 2010

14:00 – 22:00 Registration Open . . . . . Regina Isabella Hotel, Lobby

20:00 – 23:00 Welcome Reception . . . . . Regina Isabella Hotel, Poolside
Gianni Marone, Italy
Massimo Triggiani, Italy

Monday, 26 April 2010

6:30 – 13:30 Registration Open . . . . . Angelo Rizzoli Conference Centre (ARCC)

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

7:00 – 17:00 Authors Set Posters . . . . . Gallery of Posters

8:00 – 10:00 Oral Abstract Session 1: . . . . . ARCC
Genetic and Environmental Factors in Allergic Disorders
Chairpersons: Heidrun Behrendt, Germany
Stephen T. Holgate, United Kingdom

8:00 **1** *Impact of rare genetic variants on asthma and allergy susceptibility: The case of IL13R105Q*
Donata Vercelli, United States

8:17 **2** *Functional variant in vascular endothelial growth factor (VEGFA) gene is a strong predictor of airway function in children and adults*
Angela Simpson, United Kingdom—*Travel Grant Award Recipient*

8:34 **3** *Genome-wide association study of asthma susceptibility and severity*
Deborah Meyers, United States

8:51 **4** *Mutations in the fatty acid transporter protein (FATP) 4 gene induce elevated IgE, eosinophilia and atopic disorders including atopic dermatitis-like skin lesions*
Frode Jahnsen, Norway

9:08 **5** *Beyond atopy: Multiple patterns of sensitization in relation to asthma in a birth cohort study*
Adnan Custovic, United Kingdom

9:25 **6** *Asthma heterogeneity and its importance in genomics studies*
Eugene Bleecker, United States

9:42 **7** *Cigarette smoke modulates airway epithelial cell responses to human rhinovirus infection*
David Proud, Canada

10:00 – 10:30 Coffee Break. . . . . ARCC

10:30 – 11:55 Oral Abstract Session 2: . . . . . ARCC
Mast Cells
Chairpersons: Toshiaki Kawakami, United States
Dean D. Metcalfe, United States

10:30 **8** *Mast cell-derived TNF can exacerbate mortality during severe bacterial infections in C57BL/6-Kit$^{W\text{-}sh/W\text{-}sh}$ mice*
Stephen Galli, United States

10:47 **9** *The "Allergic Effector Unit": Mast cell-eosinophil activating interactions*
Francesca Levi-Schaffer, Israel

11:04 **10** *Mast cell-restricted granule proteases and tumorigenesis*
Mark Sinnamon, United States

11:21 **11** *Interleukin-33 and antigen synergistically amplify cytokine production in mast cells by co-operative interactions among signaling pathways and transcription factors*
Michael Beaven, United States

11:38 **12** *A novel point mutation of c-Kit inhibits receptor internalization, mast cell survival and development*
Silvia Bulfone-Paus, Germany

12:00 – 13:25 Oral Abstract Session 3: . . . . . ARCC
Eosinophils
Chairpersons: Junichi Chihara, Japan
Peter Weller, United States

12:00 **13** *Use of a novel IgY antibody recognizing 6'-sulfated sialyl Lewis X to identify endogenous lung ligands for Siglec-F on eosinophils*
Bruce Bochner, United States

Schedule of Events

Monday, 26 April 2010 (continued)

12:17 **14** *The involvement of lysyl tRNA synthetase in gene regulation*
Ehud Razin, Israel

12:34 **15** *New extracellular structures generated by eosinophils: Role of mitochondrial DNA*
Hans-Uwe Simon, Switzerland

12:51 **16** *Acting out of the box: Extracellular eosinophil granules are secretory competent cluster bombs*
Peter Weller, United States

13:08 **17** *Eosinophils are a major source of transforming growth factor at mucosal sites*
Stephan Bischoff, Germany

13:30 – 14:45 Lunch . . . . . Regina Isabella Hotel Restaurant

13:45 – 14:45 CIA Council Meeting . . . . . Regina Isabella Hotel, Sala Azzurra

15:00 – 19:00 Registration Open . . . . . ARCC

15:00 – 16:45 Oral Abstract Session 4: . . . . . ARCC
Mast Cells and Basophils
Chairpersons: Michael Beaven, United States
Dean Befus, Canada

15:00 **18** *Murine basophils are mature and activated upon arrival in the popliteal lymph node after papain footpad injection*
Michael Gurish, United States

15:17 **19** *Mast cells in severe asthma: Evidence for alteration in phenotype, location and associated activation*
Sally Wenzel, United States

15:34 **20** *Activated T cells release microparticles that activate mast cells*
Yoseph Mekori, Israel

15:51 **21** *Mast cells and regulatory T cells in allergic inflammation: Who regulates who and what?*
Marcus Maurer, Germany

16:08 **22** *Infections affect mast cell reactivity – possible implications for asthma exacerbations*
Gunnar Nilsson, Sweden

16:25 **23** *Vitamin D_3 promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice*
Michele Grimbaldeston, Australia

17:00 – 19:00 Poster Session 1: . . . . . Gallery of Posters
Genetic and Environmental Factors in Allergic Disorders
Chairpersons: Wayne Thomas, Australia
Donata Vercelli, United States

24 *New high-throughput strategies for systemic phenotype analysis of mutant mouse lines as models for IgE-mediated allergic diseases*
Juan Aguilar Pimentel, Germany

25 *Polymorphisms in Claudin-1 (CLDN1) and risk of asthma in independent populations of African descent*
Kathleen Barnes, United States

26 *Measuring allergens and pollen across Europe: The EU-project HIALINE*
Jeroen Buters, Germany

27 *Molecular and genetic studies around the relationship between ascariasis and asthma in humans*
Luis Caraballo, Colombia

28 *How immune peptidases change specificity: Cathepsin G gained tryptic function by missense mutation during primate evolution*
George Caughey, United States

29 *House dust mites studies in Lithuania*
Ruta Dubakiene, Lithuania

30 *Gene-environment interactions in utero: Gluthathione-S-transferase polymorphisms, prenatal exposure to acetaminophen and tobacco smoke and risk of childhood asthma*
John Holloway, United Kingdom

31 *The NPS-NPSR1 pathway in asthma and allergy: An update 2010*
Juha Kere, Sweden

Schedule of Events

Monday, 26 April 2010 (continued)

32 *Expression of innate immune receptors in cord blood leucocytes and atopic dermatitis during the first two years of life*
Roger Lauener, Switzerland

33 *Genotype determined expression of genes differentially expressed in acute childhood asthma*
Peter Le Souëf, Australia

34 *CD14 polymorphisms and serum CD14 levels through childhood; a role for gene methylation?*
Monica Munthe-Kaas, Norway

35 *Epidemiological study of specific IgE responses among junior high school students in Wakayama, Japan by using a solid phase multiplex technology*
Takemasa Nakagawa, Japan

36 *Immunologic and environmental modulation of the "Allergic March"*
Riccardo Polosa, Italy

37 *Allergen sensitization and total IgE levels during childhood differently associated with perinatal exposure to marine pollutants*
Lars Poulsen, Denmark

38 *Evaluation of Immunoglobulin G antibodies against* Aspergillus versicolor *in a group of elderly women as biomarker of mould exposure and surrogate marker of indoor situation*
Monika Raulf-Heimsoth, Germany

39 *The transgenerational asthma-preventive effect of* Acinetobacter lwoffii F78 *is mediated by innate and adaptive immune mechanisms together with epigenetic regulation*
Harald Renz, Germany

40 *The role of CD23 in IgE dependent signaling and pharmacotherapy of allergic disease*
Lanny Rosenwasser, United States

41 *Oral mite anaphylaxis (the pancake syndrome)*
Mario Sánchez-Borges, Venezuela

42 *IgG1, IgG4 and IgE antibodies to nasopharyngeal colonizing bacteria in the development of atopy and asthma*
Wayne Thomas, Australia

43 *β2-adrenergic receptor haplotypes and asthma in childhood*
Tale Torjussen, Norway

44 *Flame retardant BDE-153 stimulates adaptive immunity to inhaled allergens by activation of dendritic cells*
Leonie van Rijt, Netherlands

17:00 – 19:00 **Poster Session 2: . . . . . Gallery of Posters**
Mast Cells, Basophils, Eosinophils, Dendritic Cells and T Cells
Chairpersons: Hannah Gould, United Kingdom
Richard L. Stevens, United States

45 *The influence of isoforms of the major birch pollen antigen Bet v 1 on the activation of dendritic cells and T cell differentiation*
Gernot Achatz, Austria

46 *Inducible nitric oxide synthase expression and nitric oxide production in mouse bone marrow-derived mast cells*
Dean Befus, Canada

47 *Crosstalk of interleukin-4, stem cell factor, and immunoglobulin E-dependent activation in mature human mast cells*
Stephan Bischoff, Germany

48 *Differential T cell responses after exposure of dendritic cells to Bet v 1 isoforms or homologous food allergens*
Heimo Breiteneder, Austria

49 *IL-4 influences Th2 development via STAT6-dependent downregulation of negative acting T-Cell Factor 1 isoforms in peripheral T cells*
Albert Duschl, Austria

50 *The allergic effector unit: Mast cell-eosinophil paracrine and physical interactions regulate cell survival*
Moran Elishmereni, Israel—*Travel Grant Award Recipient*

Monday, 26 April 2010 (continued)

51 *Omalizumab is also effective in non autoimmune urticaria*
Marta Ferrer, Spain

52 *The bacterial quorum sensing molecule, N-(3-oxododecanoyl)-L-homoserine lactone, attenuates mast cell responses to stimuli via a nitric oxide dependent mechanism*
Paul Forsythe, Canada

53 *Control of human basophil activation by the SH2-containing inositol 5-phosphatase (SHIP)-1 is dependent on the nature of high-affinity IgE receptor engagement*
Bernhard Gibbs, United Kingdom

54 *Induction of airway hyperreactivity depends on a subset of iNKT cells that express IL-17RB and can be inhibited by a CD1d-dependent antagonist*
Eckard Hamelmann, Germany

55 *Endogenous IL-10 helps limit excessive Th2 responses in peanut allergic humans yet plays little role in maintaining clinical tolerance in non-allergic individuals*
Kent HayGlass, Canada

56 *A Th1/Th2-associated chemokine imbalance preceding allergic disease is influenced by birthsize, breastfeeding, daycare and probiotics*
Maria Jenmalm, Sweden

57 *Human CD8 T-cells initiate and sustain Th1 responses by promoting IL-12p70 and differentiation of blood monocytes into TNF-α/iNOS-producing dendritic cells*
David Kemeny, Singapore

58 *H3/H4 histamine receptors and IgE regulation in healthy donors and allergic patients*
Roman Khanferyan, Russia

59 *Both CD4+ and CD8+ T cells in skin of atopic dermatitis and psoriasis patient are potent cytokine releasing cells, each disease with its specific T cell cytokine profile*
Edward Knol, Netherlands

60 *CCL23 production by human eosinophils*
Kenji Matsumoto, Japan

61 *High-resolution transcriptional profiling of chemical-stimulated dendritic cells identifies immunogenic contact allergens, but not prohaptens*
Hans Merk, Germany

62 *Intracellular introduction of phosphorylated FcεRIβ immunoreceptor tyrosine-based activation motif inhibits IgE-dependent human mast cell activation*
Yoshimichi Okayama, Japan

63 *Multicistronic vector based introduction of T-cell receptor alpha and beta chains and Foxp3 or TGF-beta generates human transgenic allergen-specific T-regulatory cells*
Winfried Pickl, Austria

64 *Beneficial role for the tryptase mouse mast cell protease 6 in the innate immune response in the lung to pneumonia virus of mice*
Catherine Ptaschinski, Australia—*Travel Grant Award Recipient*

65 *Is "MCreg" a new cell in the heaven of regulatory cells?*
Carlo Pucillo, Italy

66 *Glycation of the model food allergen ovalbumin enhances antigen uptake and presentation by human dendritic cells augmenting allergen-specific Th2 cell responses*
Joachim Saloga, Germany

67 *Active transport of contact allergens in human dendritic cells and human epidermal keratinocytes is mediated by multidrug resistance related proteins*
Claudia Skazik, Germany

68 *Nickel (Ni) allergic patients with complications to Ni-containing joint replacement show preferential IL-17 type reactivity to Ni*
Burkhard Summer, Germany

69 *IL-17RB$_+$NKT cell-mediated airway hypersensitivity and* IL17RB *polymorphisms associated with severity of childhood asthma*
Masaru Taniguchi, Japan

Schedule of Events

Monday, 26 April 2010 (continued)

70 *Influence of a tumor on mast cell number and function in human lung parenchyma*
Jane Warner, United Kingdom

71 *Release of dipeptidyl peptidase I from human mast cells in anaphylaxis*
Hilary Whitworth, United Kingdom—*Travel Grant Award Recipient*

72 *The role of the eosinophil in airway remodelling in asthma*
Susan Wilson, United Kingdom

73 *Priming of human basophils by low levels of anti-FcεRI α-chain mAb*
Masao Yamaguchi, Japan

74 *Dipeptidyl peptidase I modulates the generation of active tryptase and chymase in human mast cells*
Xiaoying Zhou, United Kingdom

Tuesday, 27 April 2010

7:30 – 12:00 Registration Open . . . . . ARCC

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

8:00 – 9:30 Oral Abstract Session 5: . . . . . ARCC
T Cells and Immunoregulation
Chairpersons: Cezmi Akdis, Switzerland
Kent HayGlass, Canada

8:00 **75** *Role of the new Th22 subset in allergy*
Carsten Schmidt-Weber, United Kingdom

8:17 **76** *Tonsils are organs of immunotolerance to develop allergen-specific immunotherapy approaches targeting dendritic cells and Treg cells*
Oscar Palomares, Switzerland

8:34 **77** *Changes in regulatory T cells activity during early childhood: Novel studies using thymic tissue*
Meri Tulic, Australia—*Travel Grant Award Recipient*

8:51 **78** *Ontogeny of toll-like receptor expression and function in allergic and non-allergic children: The first 5 years*
Susan Prescott, Australia

9:08 **79** *IL-13 attenuates IL-17A production in an IL-10 dependent manner in mouse and human Th17 cells*
R. Stokes Peebles, United States

9:30 – 9:50 Coffee Break. . . . . ARCC

9:50 – 11:00 Oral Abstract Session 6: . . . . . ARCC
Regulatory Cells and Memory Stem Cells
Chairpersons: Silvia Bulfone-Paus, Germany
Guido Rossi, Italy

9:50 **80** *Identification of CD8+ memory stem cells guides to novel strategies for adoptive T cell therapy*
Dirk Busch, Germany

10:07 **81** *Innate production of Th2 cytokines by "natural helper cells"*
Shigeo Koyasu, Japan

10:24 **82** *Human natural killer cell subsets: Their potential immunoregulatory roles*
Gunnur Deniz, Turkey

10:41 **83** *Allergen-specific IL-17$^+$IL-4$^+$CD4$^+$ T cells circulate in patients with Type I allergy*
Barbara Bohle, Austria

Tuesday, 27 April 2010 (continued)

11:00 – 11:45 **Paul Kallós Lecture: . . . . . ARCC**
Inflammation, the Seventh Hallmark of Cancer

Chair: Gianni Marone, Italy

Prof. Alberto Mantovani, MD
Scientific Director
Istituto Clinico Humanitas and University of Milan
Italy

Prof. Alberto Mantovani is the Scientific Director of the Istituto Clinico Humanitas, President and founder of the Fondazione Humanitas per la Ricerca and a Professor of Pathology at the State University of Milan School of Medicine. Prof. Mantovani was born in Milan in 1948. He graduated (*summa cum laude*) in 1973 in Medicine at the University of Milan and in 1976 he specialized in Oncology at the University of Pavia. From 1973 to 1975 he had a scholarship at the Laboratory of Immunology and Chemotherapy at the Mario Negri Institute in Milano. From 1973 to 1976 he was visiting fellow at the Department of Tumor Immunology of the Chester Research Institute in Belmont (GB). In 1978-1979 he was visiting fellow at the Laboratory of Immunodiagnosis, NIH, Bethesda (USA), with a Yamagiwa-Yoshida Scholarship of the UICC first and then with a NATO grant.

From 1979 to 1981 he was Senior investigator, Department of Tumor Immunology and Chemotherapy, Istituto di Ricerche Farmacologiche "Mario Negri," Milan. In 1981 he became Chief of the Laboratory of Immunology, Istituto di Ricerche Farmacologiche "Mario Negri." From 1994 to 2001 he was full Professor of General Pathology, School of Medicine, University of Brescia, Italy. From 1996 to 2005 he has lead the Department of Immunology and Cell Biology at the Istituto di Ricerche Farmacologiche "Mario Negri," Milan.

He was appointed numerous scientific awards, i.e. Biotec award (1998); the Marie T. Bonazinga Award by the Society of Leukocyte Biology (USA) (2000); the Guido Venosta Prize by the President of the Republic of Italy (2004); EFIS – Schering Plough 1st European Immunology Prize, Paris, France (2006); Galileo Galilei Prize for Research in Biomedical Sciences (2007); PISO Award (2007); *Onorificenza al Merito della Repubblica Italiana*; the William Harvey Award (2009). Member of various professional societies, e.g. European Molecular Biology Organization (EMBO); Henry Kunkel Society; the Faculty of 1000 Biology; and President of the International Cytokine Society. More than 600 publications mostly in high-ranking journals. Highly cited immunologist, the Institute for Scientific Information (ISI Thomson) ranked him as one of 100 most quoted immunologists in the world over the last 20 years.

His research centers mainly on molecular regulation mechanisms of leukocyte infiltration. More specifically his attention is focused on the molecular function and regulation circuits of the inflammatory cytokines. The first interest was the primary inflammatory cytokine interleukin 1 (IL-1). In particular negative regulation circuits made up of type II decoy receptors. This molecule, membrane bound or released, acts as a molecular trap for the IL-1, constituting a unique system in biology. This paradigm was subsequently generalized by other inflammatory cytokines. In addition, a new IL-1 antagonist receptor was identified and defined.

In general these studies bring to light the complexity and uniqueness of IL-1negative regulation mechanisms. A second line of work centers on the study of secondary cytokines known as chemokines. New molecules have been defined (for example, macrophage derived chemokines, MDC) and new cellular targets. Among these the study of dendritic and endothelial cells has been emphasized.

In addition, the discovery that proand anti- inflammatory signals not only regulate the level of chemokine production but also the level of receptions offers a new level of understanding of how the system functions and possible targets for therapeutic interventions. Finally, his group has cloned the PTX3 (cDNA and genome, human and mouse), the first member of the long pentraxin family, and defined his function in innate immunity and inflammation.

12:00 – 18:30 **Boat Ride. . . . . Depart from Lacco Ameno Pier**

Schedule of Events

Wednesday, 28 April 2010

7:30 – 13:15 Registration Open . . . . . ARCC

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

8:00 – 9:45 Oral Abstract Session 7: . . . . . ARCC
Dendritic Cells and Macrophages
Chairpersons: Susan Prescott, Australia
Massimo Triggiani, Italy

8:00 **84** *Inhibition of Th2 adaptive immune responses and pulmonary inflammation by leukocyte Ig-like receptor B4 on dendritic cells*
Howard Katz, United States

8:17 **85** *Histamine modulates dendritic cell toll-like receptor activation via the histamine receptor 2*
Liam O'Mahony, Switzerland

8:34 **86** *A subpopulation of neutrophils induces FcεRI on mouse lung conventional dendritic cells through a CD11b dependent process*
Mitchell Grayson, United States—*Travel Grant Award Recipient*

8:51 **87** *Human dermal fibroblasts support the differentiation of IL-17 producing T-cells via up-regulation of IL-23 production by dendritic cells*
Jan Simon, Germany

9:08 **88** *Synergistic effect of secreted phospholipases A_2 and adenosine on the induction vascular endothelial growth factors in human lung macrophages*
Francescopaolo Granata, Italy

9:25 **89** *Group V secretory phospholipase A_2 modulates phagosome maturation and regulates the innate immune response against* Candida albicans
Barbara Balestrieri, United States

9:45 – 10:15 Coffee Break. . . . . ARCC

10:15 – 11:30 Oral Abstract Session 8: . . . . . ARCC
Pathophysiology of Allergic and Inflammatory Diseases
Chairpersons: Bruce Bochner, United States
George Caughey, United States

10:15 **90** *Localization of the calcitonin-gene-related peptide-associated receptor activity modifying protein 1 (RAMP1) to airway epithelial cells and its dysregulation in asthma*
A. Barry Kay, United Kingdom

10:32 **91** *Tryptase-dependent experimental colitis*
Richard L. Stevens, United States

10:49 **92** *Critical signaling nodes in mast cell proliferative and activation disorders*
Dean D. Metcalfe, United States

11:06 **93** *Regulation of tight junctions in human airway epithelium by T regulatory cells and their cytokines*
Cezmi Akdis, Switzerland

11:30 – 12:25 Oral Abstract Session 9: . . . . . ARCC
Pathophysiology of Allergic Diseases
Chairpersons: Yoseph Mekori, Israel
Hirohisa Saito, Japan

11:30 **94** *Dysregulated innate and adaptive immune responses within the airways in severe asthma and the relevance of bacteria to disease persistence.*
Peter Howarth, United Kingdom

11:47 **95** *Interactions between inflammatory pathways triggered by host responses to respiratory viruses and aeroallergens in the aetiology and pathogenesis of atopic asthma: Acute severe asthma exacerbations as a paradigm*
Patrick Holt, Australia

12:04 **96** *A selective H_4R antagonist prevents antigen-induced airway inflammation in guinea pig: A pivotal role of annexin-A1*
Emanuela Masini, Italy

Wednesday, 28 April 2010 (continued)

12:25 – 13:15 **Carl Prausnitz Lecture: . . . . . ARCC**

Mast Cells and Basophils in Angiogenesis: Bridging the Gap between Inflammation and Tumors

Chair: Stephen Galli, United States

Gianni Marone, MD FRCP
Director, Center for Basic and Clinical Immunology Research (CISI)
University of Naples Federico II
Italy

Gianni Marone is Professor of Medicine and Clinical Immunology and Director of the Centre for Basic and Clinical Immunology Research (CISI) of the University of Naples Federico II where scientists and clinicians are studying the immunological basis and pathophysiological mechanisms of allergic, inflammatory and neoplastic disorders. He is Director of the Post-graduate Training Programme in Allergy and Clinical Immunology, directs the Section of Tissue Damage of the Ruggero Ceppellini School, and is member of the Executive Board of the Centre of Excellence in Biotechnology and Biomedicine of the University of Naples Federico II.

He graduated from the School of Medicine of the University of Naples, Italy, in 1972 *summa cum laude* where he started his academic career. From 1976 to 1980 he worked at the Johns Hopkins University under the supervision of Dr. Lawrence Lichtenstein where he made contributions to the characterization of the adenosine receptors on human inflammatory cells. While in Baltimore, he contributed to the biochemical and molecular characterization of the antiinflammatory effects of glucocorticoids and the role of lipoxygenase metabolites in the release of mediators from basophils and mast cells.

In 1980, Professor Marone returned to Italy where he established his own group in the University of Naples Federico II and became Director of the Division of Clinical Immunology and Allergy. He maintained close ties with the Johns Hopkins University where he was Visiting Professor for several years and in 1998 he was elected to the Johns Hopkins Society of Scholars.

His group is at present looking into the molecular events underlying angiogenesis and lymphangiogensis in human inflammatory and neoplastic disorders. In addition, they identified the antiinflammatory effects of cyclosporins, the possible role of superallergens and secretory phospholipases in allergic and inflammatory disorders. His work has resulted in 380 articles and 18 books.

Professor Marone has been President of the Italian Society of Allergy and Clinical Immunology, and of the Italian Federation of Immunological Societies, member of the Executive Board of the UCB Institute of Allergy, and is currently President of the *Collegium Internationale Allergologicum*. He has been elected honorary member of several scientific societies among which the *Deutsche Gesellschaft für Allergologie und Klinische Immunologie*, the *Pontificia Accademia Tiberina*, the International Society of Immunorehabilitation, the Italian Society of Dermatology, and the Italian Association of Specialists in Allergy and Immunology (AISAI).

In recognition of his scientific activities he has received numerous awards: the Guido Dorso Award, the Angelo Minich Award, the RoC Award, the Esculapio Award, the *Grandi della Campania Award*, the Number One Award for Humanity in the Healthcare Profession and the 2010 IMID Award.

13:15 – 14:30 Lunch . . . . . Regina Isabella Hotel Restaurant

15:00 – 20:00 Registration Open . . . . . ARCC

15:00 – 16:45 **Oral Abstract Session 10: . . . . . ARCC**

Experimental Allergy

Chairpersons: Steve Krilis, Australia
Takemasa Nakagawa, Japan

15:00 **97** *Conformational change in IgE upon receptor binding as a basis for inhibitor design*
Brian Sutton, United Kingdom

15:17 **98** *An itch-producing sensory nerve population in the mouse*
Bradley Undem, United States

15:34 **99** *Periostin, an extracellular matrix protein, acts as a master switch for the onset of inflammation in atopic dermatitis*
Kenji Izuhara, Japan

Schedule of Events

Wednesday, 28 April 2010 (continued)

15:51 **100** *PLC-β3 deficiency causes skin inflammation*
Toshiaki Kawakami, United States

16:08 **101** *Cord blood hemopoietic progenitor cell toll-like receptor expression and function: A mechanism underlying allergic inflammation in early life?*
Pia Reece, Canada

16:25 **102** *Active immunization with mimotopes mimicking the extracellular membrane-proximal domain (EMPD) of mIgE leads to the generation of anti-mIgE antibodies* in vivo
Stefan Feichtner, Austria—*Travel Grant Award Recipient*

17:00 – 19:00 Poster Session 3: . . . . . Gallery of Posters
Experimental Models of Inflammatory and Allergic Diseases
Chairpersons: Mübeccel Akdis, Switzerland
John Bienenstock, Canada

103 *Investigating the role of osteopontin in psoriasis: Immunoregulation of antimicrobial peptides*
Anna Balato, Italy

104 *Expanding the view of allergenicity: Adjuvant, non-allergenic factors from pollen regulate disparate signaling pathways in human target cells resulting in a proallergenic micromilieu*
Heidrun Behrendt, Germany

105 *Eoxins in asthma in children - a new pathway of inflammation*
Kai-Hakon Carlsen, Norway

106 Helicobacter pylori *Hp(2-20) promotes gastric mucosal healing and interacts with formyl-peptide receptors*
Amato de Paulis, Italy

107 *Ovalbumin nitration is associated with a reduced risk for de-novo sensitization via the oral route in a murine food allergy model*
Susanne Diesner, Austria

108 *Mast cell tryptase as a mediator of inflammation through mechanisms independent of protease activated receptor 2*
Mogib El-Rahman Khedr, United Kingdom

109 *Heterogeneity of the effects of radiocontrast media on mediator release from human mast cells and basophils*
Arturo Genovese, Italy

110 *Structural damage of surfactant protein-D (SP-D) in ozone (O3)-induced exacerbation of allergic airway inflammation is associated with dendritic cell activation*
Angela Haczku, United States

111 *The activin A-binding protein follistatin inhibits airway remodeling in a murine model of chronic asthma*
Charles Hardy, Australia

112 *A pathogenic mechanism of eczema vaccinatum*
Yuko Kawakami, United States

113 *Alveolar macrophages inhibit both Th1 and Th2 immune response and induce airway remodeling in intranasally sensitized mouse asthma model*
You-Young Kim, Korea

114 *Mechanistic basis for IgE-mediated Facilitated Antigen Presentation (FAP) and inhibition by allergen-specific IgG*
Kaare Lund, Denmark

115 *The nitrosative stress in chronic nonproductive cough patients with increased nociceptive sensitivity*
Hee-Bom Moon, Korea

116 *Analysis of T cell-dependent bronchoconstriction using human cultured bronchial smooth muscle cells*
Akio Mori, Japan

117 *The participation of serine protease in the development of allergic airway inflammation in mice*
Hiroichi Nagai, Japan

118 *More than 99 percent of allergen-specific serum-IgE in the allergic patient does not originate from circulating plasma cells*
Verena Niederberger, Austria

119 *SERCA-2 expression in airway smooth muscle is altered by inflammatory cytokines and formoterol*
Oluwaseun Ojo, United Kingdom

Wednesday, 28 April 2010 (continued)

120 *Role of lung apolipoprotein A1 in development of asthma: Anti-inflammatory and anti - fibrotic effect*
Choon-Sik Park, Korea

121 *67 kDa laminin receptor (67LR) involvement in the trafficking of normal and leukemic hematopoietic stem cells; computer aided identification of a small inhibitory molecule*
Ada Pesapane, Italy

122 *Urokinase receptor (uPAR) involvement in hematopoietic stem cells homing and engraftment to bone marrow*
Ada Pesapane, Italy

123 *Expression of antimicrobial peptides in patients with COPD and detection of RSV or Influenza A*
Gernot Rohde, Germany

124 *Der p 1 exerts adjuvant function for Der p 2: Acute dermatitis in atopic and non-atopic mouse model*
Krisztina Szalai, Austria—*Travel Grant Award Recipient*

125 *The roles of phosphoinositide 3-kinase gamma for the pathogenesis of asthma and eosinophil functions*
Masahide Takeda, Japan

126 *CD8 T cells stimulate dendritic cell interleukin 12 and inhibit airway eosinophil recruitment and lung inflammation by IFN-gamma independent and dependent mechanisms*
Yafang Tang, Singapore

127 *Role of Rac2 in bleomycin-induced pulmonary fibrosis*
Harissios Vliagoftis, Canada

128 *Mimotope therapy reduces grass pollen-induced asthma in a physiologically relevant mouse model*
Julia Wallmann, Austria

17:00 – 19:00 **Poster Session 4: . . . . . Gallery of Posters**
Allergy Diagnosis *In Vitro* and *In Vivo*
Chairpersons: Fatima Ferreira, Austria
Maria Sanz, Spain

129 *Anaphylaxis to soy products in patients with birch pollinosis: Is it Gly m 4 or not?*
Jaap Akkerdaas, Netherlands

130 *Peanut allergen components in relation to DBPCFC to peanut in children with suspected peanut allergy*
Magnus Borres, Sweden

131 *A novel lipid-transfer protein of* Apium graveolens *as potential cross-reactive allergen involved in the celery-mugwort-spice syndrome*
Gabriele Gadermaier, Austria

132 *Evaluation of diagnostic potential of 1,3-beta-glucanace from banana fruit*
Marija Gavrovic-Jankulovic, Serbia

133 *The interaction between monoclonal antibodies and recombinant and natural purified Bet v 1*
Helene Henmar, Denmark

134 *Can IgE-measurement replace challenge tests in allergic rhinoconjunctivits to grass pollen?*
Johannes Huss-Marp, Germany

135 *Characterization of the T cell response to Amb a 1, the major ragweed pollen allergen and the cross-reactivity to its mugwort homologue Art v 6*
Beatrice Jahn-Schmid, Austria

136 *Pilot study on lupine allergy: cross-reactivity among legumes with and without co-existing legume allergy*
Uta Jappe, Germany

137 *Allergenic and immunotoxic activities of fungi*
Roman Khanferyan, Russia

138 *IgE-mediated diclofenac allergy: Induction mechanism and improvement of diagnosis*
Tamar Kinaciyan, Austria

139 *Component-resolved analysis of IgE antibody responses in pediatric patients allergic to domestic animals*
Jonas Lidholm, Sweden

140 *Lipids modify structure and digestibility of peanut allergen Ara h 8*
Arnd Petersen, Germany

141 *X-ray crystallographic mapping of antigenic determinants in Bla g 2 using non-overlapping monoclonal antibodies*
Anna Pomés, United States

Schedule of Events

Wednesday, 28 April 2010 (continued)

142 *The very limited usefulness of skin testing with penicilloyl-polylysine and the minor determinant mixture in evaluating nonimmediate reactions to penicillins*
Antonino Romano, Italy

143 *Assessment of cut off points for pollen allergens represented in ISAC microarray CRD103*
Maria Sanz, Spain

144 *Heparin associated dermal necrosis as manifestation of heparin induced thrombocytopenia II?*
Jan Simon, Germany

145 *Recombinant allergens in the diagnosis of pollen-allergic patients: Comparison with natural allergens extracts*
Giuseppe Spadaro, Italy

146 *Allergen sensitization of infants and 1-6 years old children in Debrecen (Hungary) within 2004-2006 years*
Paul Szemere, Hungary

147 *Enzymatic characterization of house dust and storage mite allergen extracts*
Betty Goedewaagen, Netherlands

148 *Commercial* Dermatophagoides pteronyssinus *extracts show a great variability regarding the allergen content and cutaneous responses*
Susanne Vrtala, Austria

149 *Identification and characterization of a conformational epitope on the cashew nut 11S globulin allergen, Ana o 2*
LeAnna Willison, United States

150 *Increased allergen-specific IgE titers are linked to increased diversity of the serum IgE composition*
Peter Würtzen, Denmark

151 *Component resolved diagnosis (CRD) for hazelnut allergy in EuroPrevall*
Laurian Zuidmeer, Netherlands

152 *Identification and immunoreactivity of almond lipid transfer protein, Pru du 3*
LeAnna Willison, United States

19:00 – 20:00 **Relaxing from Immunology** (accompanying persons included) . . . . . **ARCC**
Deadly Germs and Immortal Music

Chair: Johannes Ring, Germany

Prof. Dr. Dr. h. c. Ernst Th. Rietschel
Leibniz Association
Germany

Ernst Theodor Rietschel was born in Gießen (Germany) in 1941. After receiving his early education in Frankfurt, he studied chemistry at the Ludwig-Maximilians-University in Munich (1961-1963) and the Albert-Ludwigs-University in Freiburg (1968). During his diploma work (1968) he elucidated the crystal structure of SrI2.

In the same year he was offered a fellowship of the Stiftung Volkswagenwerk and entered the Max-Planck-Institute for Immunobiology in Freiburg where he received his PhD graduation with Otto Westphal (1971). Thereafter, he worked as a post-doctoral fellow at the University of Minnesota (Minneapolis) in the laboratory of D. W. Watson (1972-1973).

In the following years he again joined the Max-Planck-Institute in Freiburg working on the chemistry and biology of bacterial endotoxins. After his habilitation (1978) he accepted in 1980 a chair and full professorship for Immunochemistry and Biochemical Microbiology at the University of Lübeck. At the same time he was appointed Director of the Research Center Borstel, Leibniz-Center for Medicine and Biosciences, where he headed the Department of Immunochemistry and Biochemical Microbiology by 2006.

In 2003 he became Scientific Vice President of the Leibniz Association and in 2005 he was elected as President of the Leibniz Association. In 2006 he was assigned Acting Chair of the European Medical Research Councils (EMRC) of the European Science Foundation (ESF) and is a Member of the Council of the STS-Forum.

20:15 – 23:00 **Pizza and Pasta Party** . . . . . **Hotel Continental Terme**
Buses will depart from the Regina Isabella Hotel at 20:15.

Schedule of Events

Thursday, 29 April 2010

7:30 – 13:00 Registration Open . . . . . ARCC

7:30 - 17:00 Speaker Preview Room Open . . . . . ARCC

8:00 – 9:45 Oral Abstract Session 11: . . . . . ARCC
Allergy Diagnosis
Chairpersons: Robert Aalberse, Netherlands
Harald Renz, Germany

8:00 **153** *Structural method development on biological reference preparations: GMP-produced recombinant Phl p 5.0109 as a model allergen in different formulations*
Martin Himly, Austria

8:17 **154** *Peanut allergy: Clinical and immunological differences among patients from three different geographical regions*
Marianne van Hage, Sweden

8:34 **155** *Molecular mapping of surface exposed epitopes on the Group 1 mite allergens: Towards the resolution of the cross-reactive "Heymann" epitope*
Martin Chapman, United States

8:51 **156** *A monomeric, low IgE binding Bet v 1 derivative produced by epitope grafting provides an excellent candidate for birch pollen IT*
Michael Wallner, Austria—*Travel Grant Award Recipient*

9:08 **157** *High-throughput NMR authentication of food allergens*
Karin Hoffmann-Sommergruber, Austria

9:25 **158** *Hymenoptera venomics: A systematic approach to solve current limitations in diagnosis and treatment of insect venom allergy*
Markus Ollert, Germany

9:45 – 10:15 Coffee Break. . . . . ARCC

10:15 – 11:30 Oral Abstract Session 12: . . . . . ARCC
Genetic and Clinical Aspects of Inflammatory Disorders
Chairpersons: Franklin Adkinson Jr., United States
Alexander Kapp, Germany

10:15 **159** *Molecular genetics of eczema and atopy*
Johannes Ring, Germany

10:32 **160** In silico *identification of hair root tissue-specific gene expression profiles of atopic dermatitis*
Hirohisa Saito, Japan

10:49 **161** *Tissue oriented approach to eosinophilic esophagitis*
Oral Alpan, United States

11:06 **162** *Budesonide as induction treatment for active eosinophilic esophagitis in adolescents and adults: A randomized, double-blind, placebo-controlled study*
Alex Straumann, Switzerland

11:30 – 13:00 Oral Abstract Session 13: . . . . . ARCC
Clinical Aspects of Allergic Disorders
Chairpersons: Lanny Rosenwasser, United States
Sally Wenzel, United States

11:30 **163** *Inhaled interferonß in the treatment of exacerbations of asthma, COPD and H1N1 variant influenza*
Stephen Holgate, United Kingdom

11:47 **164** *Distinct mechanisms for bronchoprotective and anti-inflammatory effects of endogenous prostaglandin E_2*
Sven-Erik Dahlén, Sweden

12:04 **165** *Rash oral immunotherapy for the treatment of hens egg- and cows milk-induced anaphylaxis*
Motohiro Ebisawa, Japan

12:21 **166** *The World Allergy Organization survey on global availability of essentials for the assessment and management of anaphylaxis*
F. Estelle Simons, Canada

12:38 **167** *Allergies have a socioeconomic impact: A model calculation and what allergologists can do to save costs*
Torsten Zuberbier, Germany

Schedule of Events

Thursday, 29 April 2010 (continued)

13:00 – 14:15 Lunch . . . . . Regina Isabella Hotel Restaurant

16:00 – 18:45 Registration Open . . . . . ARCC

16:00 – 16:45 CIA Business Meeting . . . . . ARCC
All CIA Members are asked to attend.

17:00 – 18:45 Poster Session 5: . . . . . Gallery of Posters
Clinical Aspects of Allergic Disorders
Chairpersons: Oscar L. Frick, United States
Torsten Zuberbier, Germany

168 *Human B regulatory cells. Do they really exist?*
Mübeccel Akdis, Switzerland

169 *Nasal polyp polyclonal IgE is functional in response to allergen and SEB*
Claus Bachert, Belgium

170 *Reduced expression of claudin-1 in the epidermis of atopic dermatitis subjects disrupts barrier function and enhances susceptibility to HSV-1 infections*
Lisa Beck, United States

171 *Asthma prediction in school children: The value of combined IgE-antibodies and obstructive airways disease severity score*
Karin Carlsen, Norway

172 *Rupatadine improves nasal symptoms, airflow and inflammation in patients with persistent allergic rhinitis: A pilot study*
Giorgio Ciprandi, Italy

173 *Resistin-like molecule-β promotes human airway fibroblast cellular proliferation and differentiation and may be involved in airway fibrosis*
Chris Corrigan, United Kingdom

174 *Increased release of cysteinyl-leukotrienes in ex vivo stimulated sputum cells from subjects with aspirin-intolerant asthma*
Barbro Dahlén, Sweden

175 *Psychosomatic aspects in allergy: Organically unexplained symptoms in allergy inpatients*
Ulf Darsow, Germany

176 *Evidence of receptor revision and class switching to IgE in nasal polyps*
Philippe Gevaert, Belgium

177 *Expression of CD30+ and CD45RO+ in acute atopic dermatitis*
Ruzica Jurakic Toncic, Croatia

178 *Clarithromycin reduces disease burden after an acute asthma exacerbation. An open randomized trial*
Nikos Papadopoulos, Greece

179 *Treatment of severe asthma and food IgE-mediated diseases with anti-immunoglobulin E antibody*
Vincenzo Patella, Italy

180 *Environmental contact allergens in adult atopic dermatitis*
Györgyi Pónyai, Hungary

181 *Immunoglobulin free light chains in rhinitis*
Frank Redegeld, Netherlands

182 *Active transport of Bet v 1 allergen through nasal epithelium*
Risto Renkonen, Finland

183 *TGFß receptor expression is reduced in the airways in mild asthma compared to non-asthmatic controls*
Douglas Robinson, Spain

184 *Topical lysine aspirin in aspirin exacerbated respiratory disease*
Glenis Scadding, United Kingdom

185 *Food allergy related quality of life in patients with birch associated food allergy*
Regina Treudler, Germany

Thursday, 29 April 2010 (continued)

17:00 – 18:45 **Poster Session 6: . . . . . Gallery of Posters**
Treatment of Immune Disorders
Chairpersons: Adnan Custovic, United Kingdom
Ronald van Ree, Netherlands

186 *Hypoallergenic derivatives of the major birch pollen allergen Bet v 1 obtained by rational sequence reassembly*
Raffaela Campana, Austria

187 Phleum pretense *depigmented allergoids contain the same allergen profile as native extracts*
Jeronimo Carnes, Spain

188 *Protection of the respiratory tract by an oral killed non-typeable haemophilus influenzae vaccine*
Robert Clancy, Australia

189 *Allergen-specific IgG antibodies for treatment and prevention of type I allergic reactions*
Sabine Flicker, Austria

190 *Peptides derived from the major grass pollen allergen Phl p 5 lacking IgE reactivity and by-passing T cell recognition induce allergen-specific protective IgG responses*
Margarete Focke-Tejkl, Austria

191 *Generation of Cetuximab-like canine IgE and IgG antibodies for comparative oncology*
Erika Jensen-Jarolim, Austria

192 *A mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides and a probiotic strain suppresses the allergic sensitization against whey protein in mice*
Leon Knippels, Netherlands

193 *A peanut allergoid with reduced IgE-binding and maintained immunogenicity*
Stef Koppelman, Netherlands

194 *A Bet v 1 peptide vaccine by-passing T cells and IgE*
Birgit Linhart, Austria

195 *Alpha-MSH and fragments: Mediators of neuroinflammation with a therapeutic potential*
Thomas Luger, Germany

196 *Vaccination with carrier-bound non-allergenic Bet v 1-derived peptides induces allergen-specific IgG in a non-allergic individual*
Katharina Marth, Austria

197 *Immunological changes associated with sublingual immunotherapy efficacy*
Philippe Moingeon, France

198 *A cat vaccine based on carrier-bound Fel d 1-derived peptides for by-passing allergen-specific IgE and T cell reactivity*
Katarzyna Niespodziana, Austria

199 *Allergic responses in experimental autoimmune encephalomyelitis*
Rosetta Pedotti, Italy—*Travel Grant Award Recipient*

200 *ZaBeCor pharmaceuticals begins a phase II clinical trial with excellair in asthma patients*
Alan Schreiber, United States

201 *Biologics targeting T cells and B cells improve atopic eczema*
Dagmar Simon, Switzerland

202 *A novel role of human polyclonal IgG in the maintenance of vascular function in humans*
Giuseppe Spadaro, Italy

203 *A universal concept for the generation of hypoallergenic fish vaccines*
Ines Swoboda, Austria

204 *IgE as antitumor adjuvant: Towards a safe human vaccine based on a recombinant MVA encoding for a mini membrane IgE*
Luca Vangelista, Italy—*Travel Grant Award Recipient*

205 *The effects of immunotherapy on blood dendritic cells in patients with hymenoptera venom allergy*
J. Christian Virchow, Germany

19:00 – 24:00 **Gala Dinner . . . . . Regina Isabella Hotel Restaurant**

Schedule of Events

Friday, 30 April 2010

8:00 – 9:00 CIA Council Meeting . . . . . Regina Isabella Hotel, Sala Azzurra

8:30 – 13:00 Registration Open . . . . . ARCC

8:30 – 13:00 Speaker Preview Room Open . . . . . ARCC

9:00 – 10:30 Oral Abstract Session 14: . . . . . ARCC
Emerging Aspects in Allergic Inflammation
Chairpersons: A. Barry Kay, United Kingdom
Francesca Levi-Schaffer, Israel

9:00 **206** *Stressed airway epithelial cells release a nuclear alarmin IL-33 and trigger Th2-type immune responses*
Hirohito Kita, United States

9:17 **207** *NGF is an essential survival factor for bronchial epithelial cells during respiratory syncytial virus infection*
Giovanni Piedimonte, United States

9:34 **208** *IL-25 in asthma: A potential role in angiogenesis*
Ying Sun, United Kingdom

9:51 **209** *Intestinal mast cell levels determine oral antigen-induced anaphylaxis severity*
Simon Hogan, United States—*Travel Grant Award Recipient*

10:08 **210** *FcεRI subunit expression pattern: Functional implication in IgE-mediated intestinal disorders*
Eva Untersmayr, Austria

10:30 – 11:00 Coffee Break. . . . . ARCC

11:00 – 12:45 Oral Abstract Session 15: . . . . . ARCC
Immunotherapies
Chairpersons: Peter Howarth, United Kingdom
Mark Larché, Canada

11:00 **211** *TGF-beta and functional regulatory T cells in effective sublingual immunotherapy for house dust mite allergy*
Robyn O'Hehir, Australia

11:17 **212** *Targeting the MHC class-II antigen presentation pathway as a novel vaccination strategy for allergy*
Reto Crameri, Switzerland

11:34 **213** *Specific immune therapy during pregnancy inhibits allergic sensitization and airway disease in offspring mice*
Eckard Hamelmann, Germany

11:51 **214** *Tolerogenic dendritic cells induce CD4+CD25+Foxp3+ T regulatory cell differentiation from CD4+CD25-Foxp3- T effector cells*
John Gordon, Canada

12:08 **215** *Non-IgE blocking antibodies: Surrogate biomarkers of clinical efficacy to grass pollen immunotherapy*
Mohamed Shamji, United Kingdom

12:25 **216** *Induction of cytolytic CD-4+ T cells to soluble antigens and their potential for antigen-specific immunosuppression*
Jean-Marie Saint-Remy, Belgium

ORAL ABSTRACT SESSION 1: Genetic and Environmental Factors in Allergic Disorders

1

Impact of rare genetic variants on asthma and allergy susceptibility: The case of IL13R105Q

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The genetic determinants of susceptibility to asthma and allergy are far from understood. Analysis of common polymorphisms has identified only a limited amount of the heritable component of these complex diseases, and the factors underlying the missing heritability are unclear. Using as a model IL13, one of the most replicated asthma/allergy susceptibility genes, we are exploring the Common Disease, Rare Variant hypothesis according to which multiple rare variants of medium-high penetrance, typically residing in coding regions, are major contributors to disease susceptibility. To test this hypothesis we are using a combination of resequencing-based rare variant discovery and quantitative functional analysis of the variants thus identified.

We resequenced the IL13 exons in DNA from 96 non-Hispanic White and 96 African American asthmatics. No rare variant was identified in non-Hispanic Whites. In contrast, among African Americans we found three novel variants, one of which (IL13R105Q, minor allele frequency 1.6%) is non-synonymous and results in the non-conservative replacement of arginine (R) 105 with glutamine (Q). R105 is immediately adjacent to a 5-amino acid stretch which forms a contact site for IL-13Rα1 and is completely conserved in mice, dogs and cows. To test for biological activity, IL-13R105Q and wild type (WT) IL-13 were expressed in recombinant form in eukaryotic cells. IL-13 concentrations were assessed by ELISA using a correction factor that adjusts for the ability of anti-WT IL-13 antibodies to detect IL-13R105Q. The biological activities of WT IL-13 and IL-13R105Q were directly compared by incubating human peripheral blood mononuclear cells with WT IL-13 and IL-13R105Q (3-2,000 pg/ml) for 48 hrs and assessing IL-13-induced CD23 expression and STAT6 phosphorylation in monocytes using immunofluorescence. Both IL-13 proteins were active in the physiologic pg range, but IL-13R105Q was significantly more active than WT IL-13 in both assays.

These results show the R105Q replacement leads to profound gain-of-function alterations of IL-13 biological activity. While the mechanisms underlying the increased potency of IL-13R105Q require further investigation and genotyping in well phenotyped populations is still in progress, our data strongly suggest the rare IL13R105Q variant may significantly enhance IL13-dependent events in vivo, thereby modifying susceptibility to allergic inflammation.

2

Functional variant in vascular endothelial growth factor (VEGFA) gene is a strong predictor of airway function in children and adults

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Background: Genetic factors contribute to the regulation of airway growth. Given the role of VEGF in lung development, we hypothesized that polymorphisms in *VEGFA* may be associated with lung function. We therefore assessed the role of genetic variants in *VEGFA* as determinants of airway function in a population-based birth cohort (Manchester Asthma and Allergy Study) and a population-based sample of adults. We then proceeded to investigate the functionality of the SNP.

Methods: We measured specific airway conductance (sG_{aw}; plethysmography) at age 3 years (n=462), and FEV_1 at ages 5 (n=650) and 8 (n=632) using spirometry. The replication sample comprised 800 adults aged 28-61 years (343 males) in whom lung function was measured using spirometry. We genotyped haplotype tagging SNPs in VEGF (rs833068, rs833070, rs10434, rs3025028 and rs2146323) using single base extension method (Sequenom).

Results: At all ages (3, 5 and 8 years), airway function was significantly higher among CC homozygotes for rs3025028 compared to the CG/GG group ($p \leq 0.03$). The effect persisted beyond childhood; in adults FEV_1/FVC ratio was significantly higher in the CC (80.8%±8.6%) than the CG/GG group (79.6%±6.5%, p=0.03). Since SNP rs3025028 is located between exons 7 and 8, we hypothesized that it might alter splicing and affect the balance between the inhibitory (VEGFA-165b) and active (VEGFA-165a) isoforms. We measured VEGFA-165b and total VEGFA-165 levels (Western blot) in serum from adults selected based on genotype (16 CC, 13 GG); the CC group had significantly increased VEGFA-165b/VEGFA-165 ratio compared to the GG group (p=0.007). Amongst children, the VEGFA-165b/VEGFA-165 ratio was higher at birth (cord plasma) amongst CC (n=24) vs. GG (n=22) ($p<0.001$). sG_{aw} was significantly correlated with VEGFA-165b/VEGFA-165 ratio at age 3 and 8 years.

Conclusions: We report for the first time significant associations between *VEGFA* SNP rs3025028 and parameters of airway function measured throughout childhood in a large population-based birth cohort, with the effect persisting into adulthood. We propose that the mechanism of this effect is mediated through the ratios of active and inhibitory isoforms of VEGFA-165, which may be determined by alternative splicing.

3

Genome-wide association study of asthma susceptibility and severity

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Background: Asthma is a heterogeneous disease that is caused by the interaction of genetic susceptibility with environmental influences. Genome-wide association studies (GWAS) represent a powerful approach to investigate the association of DNA variants with disease susceptibility. To date, few GWAS for asthma susceptibility and none for asthma severity have been reported.

Objective: To identify genes involved in asthma susceptibility and in the pathogenesis of severe asthma in The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study.

Abstracts

Methods: 292,443 SNPs were tested for association with asthma in 473 TENOR cases and 1,892 Illumina general population controls. Asthma-related quantitative traits (total serum IgE, FEV_1, FVC, and FEV_1/FVC) were also tested in identified candidate regions in 473 TENOR cases and 363 phenotyped controls without a history of asthma to further analyze our GWAS results. Further analyses were performed for asthma severity using a proportional odds model in asthmatics stratified into mild, moderate, and severe groups based on NAEPP guidelines. For replication, FEV1 was analyzed in non-Hispanic White and African American asthmatics from the NHLBI Severe Asthma Research Program (SARP) and the Wake Forest Collaborative Study on the Genetics of Asthma (CSGA).

Results: Multiple SNPs in the *RAD50-IL13* region on chromosome 5q31.1 were associated with asthma: rs2244012 in intron 2 of *RAD50* (P = 3.04E-07). The HLA-DR/DQ region on chromosome 6p21.3 was also associated with asthma: rs1063355 in the 3' UTR of *HLA-DQB1* (P = 9.55E-06). Rs4569733, in intron 1 of *TMEM154* on chromosome 4q31.3, was associated with asthma severity (P = 7.26E-08, Bonferroni adjusted P = 0.023). This SNP was also associated with FEV_1 (P = 0.0026) in TENOR, and weakly replicated in non-Hispanic Whites, but not in African Americans from the other populations. Our results suggest that genes important in asthma susceptibility may differ from the modifier genes influencing asthma severity.

4

Mutations in the fatty acid transporter protein (FATP) 4 gene induce elevated IgE, eosinophilia and atopic disorders including atopic dermatitis-like skin lesions

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Background: Ichthyosis premature syndrome (IPS) is a rare autosomal recessive congenital ichthyosis caused by mutations in the FATP4 gene, which encodes a transporter and activator of long fatty acids. FATP4 is expressed in the epidermis and FATP4-deficient mice have an impaired skin barrier, suggesting that the composition of lipids is important for the barrier function. Several genetic diseases with barrier abnormalities have been closely linked to the development of atopy.

Material and methods: IPS patients were clinically examined (n=14). Blood samples and skin biopsies were obtained. Electron microscopy, histology and immunohistochemistry were performed. Global expression profiles of mRNA isolated from epidermal tissue were performed. Skin from wild type and FATP4 -/- mice and FATP4 -/- skin grafted onto nude mice was examined.

Results: 11 out of 14 IPS patients suffered from one or more allergic disorders including allergic rhinitis, asthma, and allergic reactions to food. Eosinophils were increased in all patients (mean 17% of total leukocyte counts, range 5-48) and total serum IgE was increased in 60% of the patients (mean 430 IU/ml, range 20-21652). Skin samples showed morphological changes previously described for IPS and revealed variable chronic dermal inflammation with many mast cells. Skin samples from a stillborn fetus (32 weeks) also showed dermal inflammation with many eosinophils. Similarly, fetal skin of FATP4-deficent mice showed inflammatory infiltrates and FATP4-deficent skin grafted onto nude mice showed persistent inflammation. Transcription profiling of IPS epidermis revealed increased mRNA levels of several anti-microbial proteins, which also are chemotactic for mast cells and other leukocytes.

Conclusion: We demonstrated that IPS is strongly associated with atopic manifestations. Importantly, the proinflammatory microenvironment in the skin was established in utero; independently of the external environment and the adaptive immune system. The Th2-promoting cytokine TSLP is considerably upregulated in fetal skin and serum of FATP4-deficient mice (Demehri et al. PLoS Biol. 2008; 27:123). It is therefore tempting to speculate that skin-derived production of TSLP drives the pathology in IPS. Thus, impaired barrier function caused by altered composition of lipids in the skin could be a driving force in atopic development.

5

Beyond Atopy: Multiple patterns of sensitization in relation to asthma in a birth cohort study

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Background: In epidemiological studies and clinical practice, individuals are classified as atopic if they have a positive IgE or skin prick test (SPT). We hypothesize that rather than relying on the presence or absence of IgE, more useful information may be obtained by identifying underlying patterns that characterize IgE responses in both serum and skin testing, taking into account the timing and type of sensitization to specific allergens. Such clustering may reflect different latent atopic vulnerabilities which are related to the presence of asthma in a fundamentally different way from current definition of atopy.

Methods: Within the setting of a population-based birth cohort, in which measures of allergic sensitization (sIgE and SPT) to multiple inhalant and food allergens have been taken throughout childhood, we took a machine learning approach to the data analysis. Using Bayesian inference methods (Hidden Markov Model), all available SPTs and sIgEs were used to infer one multinomial latent variable per child to cluster the children in an unsupervised manner into different sensitization classes. We went on to examine relationships between these latent classes and clinical expression of asthma (symptoms, lung function, airway reactivity).

Results: A five-class model indicated a complex latent structure, in which children with latent atopic vulnerability were clustered into four distinct classes (Multiple Early [116/1053, 11%]; Multiple Late [154/1053, 14.6%]; Predominantly Dust Mite [167/1053, 15.9%]; and Predominantly Pollen [40/1053, 3.8%]), with a fifth class describing children with No Latent Vulnerability [576/1053, 54.7%]. The associations with clinical outcomes were significantly stronger for Multiple Early compared to others classes or conventionally defined atopy. Lung function and airway reactivity (sGaw age 3 and 5, spirometry and methacholine challenge, age 8 years) were significantly poorer amongst children in Multiple Early compared to other latent classes. There was a substantial increase in the risk of hospital admission with asthma amongst children in the Multiple Early class (OR 11.2, 95%CI 4.0-31.5, P<0.0001), with no significant increase amongst those in other classes.

Conclusions: IgE antibody responses do not reflect a single phenotype of atopy, but several different latent atopic vulnerabilities which differ in their relationship with asthma presence and severity.

6

Asthma heterogeneity and its importance in genomics studies

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RATIONALE: Our research group recently completed an in-depth phenotypic analysis of Severe Asthma Research Program NHLBI (SARP) participants based on physiologic variables and ranked ordinal variables of symptoms, health care, and medications. (Moore WC, et al. AJRCCM, 2009, in press). This study showed that unsupervised cluster analysis defined unique phenotypic asthma subgroups. Polymorphisms in the IL4 and GATA3-pathway have been associated with atopy and asthma but susceptibility. However, the role of this pathway in asthma severity is not well understood. The purpose of this initial analysis was to evaluate the contribution of IL4 and GATA3 pathway genes in composite and atopic asthma clusters.

METHODS: Non-Hispanic whites from SARP with complete information on ranked ordinal variables were analyzed for SNPs in GATA3 and IL4Ra using whole-genome SNP data. All polymorphisms analyzed had a genotyping frequency > 95%, a minor allele frequency > 5%, and were in Hardy-Weinberg equilibrium ($p > 0.01$). Additive genetic associations were evaluated using chi-square tests for trend and ordinal regression.

RESULTS: For IL4Ra, the frequency of the C allele at E375A, which was previously associated with asthma severity and exacerbations (Wenzel AJRCCM, 2007), increased with atopic asthma severity (overall $p=0.009$) but was not significant in the two less atopic phenotypic clusters. Eight GATA3 polymorphisms in the 5' proximal promoter (linkage disequilibrium block) were significantly associated with increasing use of inhaled and oral corticosteroids. The strongest association was for the polymorphism rs1271899 (adjusted $p < 0.001$). SNPs in this region were also associated with increased sinus infections ($0.006 \leq p \leq 0.046$) and atopic asthma clusters of increasing severity ($0.002 \leq p \leq 0.038$).

CONCLUSION: Cluster classification of asthma is more informative than traditional mild, moderate and severe assessments. These asthma severity subgroups appear to have different genetic, molecular and physiologic phenotypes using GWAS results from our genomic studies.

7

Cigarette smoke modulates airway epithelial cell responses to human rhinovirus infection

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Human rhinovirus (HRV) infections are a major trigger for exacerbations of asthma and COPD. The airway epithelial cell is the primary site for HRV infection, and upon infection with HRV, epithelial cells produce numerous chemokines, cytokines and host defense molecules. Cigarette smoking profoundly effects airway biology and is the major cause of COPD. Moreover, about 25% of asthmatics smoke, and these individuals have more frequent hospitalizations and decreased quality of life compared to non-smoking asthmatics. We hypothesized that cigarette smoke modulates epithelial responses to HRV in a manner that would be expected to lead to worse clinical outcomes. We examined the effects of cigarette smoke extract (CSE) on responses of human bronchial epithelial cells to infection with purified HRV-16. We monitored induction of the chemokines, CXCL10 and CXCL8, and of the host defense proteins, human β-defensin-2 (HBD-2) and viperin.

Both CSE and HRV-16 induced CXCL8 production and, when used in combination, induced at least an additive production of CXCL8 compared to either stimulus alone. By contrast, CSE did not induce CXCL10 and markedly inhibited HRV-16-induced CXCL10 production. Inhibition of HRV-16-induced CXCL10 by CSE was mediated, at least in part, via transcriptional regulation, while the increased CXCL8 production seen with the combination of CSE and HRV-16 was not due to transcriptional regulation but was associated with CXCL8 mRNA stabilization. CSE did not induce expression of HBD-2 or viperin. HRV-16-induced production of viperin and this was inhibited by CSE. Although HRV-16 induces moderate expression of HBD-2, we have previously reported that this is synergistically enhanced by IL-17. Induction of HBD-2 by HRV-16, IL-17 or the combination, was also inhibited in the presence of CSE. Preliminary data using viperin promoter-luciferase constructs, suggest that CSE inhibits viperin expression, at least in part, via transcriptional regulation.

If the effects of CSE were reproduced *in vivo*, differential modulation of HRV-16-induced CXCL8 and CXCL10 by CSE would be expected to lead to enhanced airway neutrophilia and reduced recruitment of antiviral T-cells and Natural Killer cells. Reduced production of viperin and HBD-2 would also weaken host antiviral responses and contribute to worse clinical outcomes.

ORAL ABSTRACT SESSION 2: Mast Cells

8

Mast cell-derived TNF can exacerbate mortality during severe bacterial infections in C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice

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The factors determining whether particular infections will be successfully controlled or progress to death are incompletely understood. Studies conducted using genetically mast cell-deficient (WB/ReJ x C57BL/6)F_1-*Kit*$^{W/W\text{-}v}$ mice (WBB6F_1-*Kit*$^{W/W\text{-}v}$ mice), the corresponding normal (WBB6F_1-*Kit*$^{+/+}$) mice, and mast cell-engrafted WBB6F_1-*Kit*$^{W/W\text{-}v}$ mice have indicated that mast cells can increase survival during various models of bacterial infection of moderate severity, defined herein as infections that result in relatively low mortality in normal mice. We used mast cell-engrafted genetically mast cell-deficient C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice to investigate the roles of mast cells and mast cell-derived TNF in two models of severe bacterial infection. We confirmed, using C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, findings derived from studies of WBB6F_1-*Kit*$^{W/W\text{-}v}$ mice indicating that mast cells can promote survival in cecal ligation and puncture (CLP) of moderate severity (defined as a CLP procedure that results in < 50% mortality). However, we found that the beneficial role of mast cells in this setting can occur independently of mast cell-derived TNF. By contrast, using mast cell-engrafted C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$

mice, we found that mast cell-derived TNF can increase mortality during severe CLP (defined as a CLP procedure that results in > 50% mortality) and can also enhance bacterial growth and hasten death after intra-peritoneal inoculation of *Salmonella typhimurium*. In WBB6F$_1$-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, mast cells enhanced survival during moderate CLP but did not significantly change the survival observed in severe CLP. Our findings in three types of genetically mast cell-deficient mice thus support the conclusion that, depending on the circumstances (including mouse strain background, the nature of the mutation resulting in a mast cell deficiency, and type and severity of infection), mast cells can have either no detectable effect or opposite effects on survival during bacterial infections, e.g., promoting survival during moderate CLP associated with low mortality but, in C57BL/6-*Kit*$^{W\text{-}sh/W\text{-}sh}$ mice, increasing or hastening mortality during severe CLP or after intra-peritoneal inoculation with *S. typhimurium*.

9

The "Allergic Effector Unit": Mast cell-eosinophil activating interactions

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Allergic inflammation (AI) is a complex phenomenon consisting usually of two main phases, early and late. Mast cells (MC) have been historically associated with the acute early responses, while eosinophils (Eos) with the late/chronic ones. Yet both cells co-exist in the inflamed tissue in these later phases and could thus interact. Many Eos-derived soluble mediators can affect MC functions and vice versa. Beyond this paracrine communication, cell-cell contact between MCs and Eos may also occur. Despite this, little is known regarding either mechanisms of this MC/Eos "cross-talk" or its biological consequences. We hypothesize that soluble and physical interactions between MCs and Eos, i.e. the Allergic Effector Unit (AEU), exist, are functional, and can contribute to the perpetuation and severity of AI. To study the AEU and its mechanisms, we carried out co-cultures of human cord blood derived MCs and peripheral blood Eos at various ratios and under different conditions. This allowed us to examine the kinetics, morphological features, and functional profiles of both cells in concert with one other. Using time-lapse microscopy we observed that MCs and Eos formed stable couples within 1-5 minutes, and that cell membrane structures underwent visible changes. The cells moved toward each other, with MCs actively searching for Eos using pseudopod-like structures. Electron microscopy (EM) imaging confirmed tight interactions of the cells along several membrane areas. Lipid body content and granule morphology of co-cultured MCs and Eos (respectively) were altered, indicating reciprocal activation. Indeed, mediator assays revealed that MCs activate Eos to release Eos peroxidase (EPO), while Eos induce MC degranulation and release of tryptase. These mediators also underwent transfer from their cell of origin to the other cell, as shown by immunostaining and EM. Another Eos mediator, major basic protein (MBP) was also found to be uptaken by MCs in a time-dependent fashion, as shown by intracellular flow cytometry. This is the first morphological and functional study characterizing a human MC-Eos cross-talk in vitro. Identification of AEU-associated therapeutic targets could promote translational research aimed at ameliorating AI diseases.

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Mast cell-restricted granule proteases and tumorigenesis

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Introduction: Inflammation is associated with the development of cancer. In some solid tumors, the presence of mast cells (MCs) is associated with a dire clinical prognosis. Chymase-1 and tryptase-β are the two major proteases in human MCs, and their orthologs are mouse MC protease (mMCP)-5 and mMCP-6, respectively. The constitutive MCs in the skin and lungs express mMCP-5 and mMCP-6, and we discovered that the transcripts that encode these proteases are present in murine intestinal adenomas. The K-ras^{LA1} mouse develops spontaneous lung adenomas and adenocarcinomas. Because we noticed MCs at the margins of these tumors, we combined this cancer model and an orthotopic fibrosarcoma model using mice deficient in MCs, mMCP-5, or mMCP-6 to explore the biological significance of MC-restricted proteases in tumorigenesis.

Methods: We compared C57BL/6 (B6) K-ras^{LA1}, K-ras^{LA1}-W^{sh}/W^{sh}, K-ras^{LA1}-mMCP-6$^{-/-}$; and K-ras^{LA1}-mMCP -5$^{-/-}$ mice. We euthanized the animals at 6, 9, and 14 weeks and processed their lungs for tumor counts and volume fraction, histopathology, and transcript expression. In the second cancer model, mMCP-5$^{-/-}$, mMCP-6$^{-/-}$, and wild-type (WT) B6 mice received a subcutaneous injection of 1x10^5 MC17-51 cells derived from a methylcloranthrene-induced fibrosarcoma. We measured tumor onset and growth daily. On day 21, we processed the tumors for RNA and histological analyses.

Results: Lung tumor numbers were slightly decreased at 6 weeks in MC-, mMCP-5-, and mMCP-6-deficient K-ras^{LA1} mice relative to control mice. On week 9, the differences in tumor numbers were significant between K-ras^{LA1} (34.3±6.3) mice and K-ras^{LA1}-W^{sh}/W^{sh} (9.2±1.2), K-ras^{LA1}-mMCP-6$^{-/-}$ (8.2±1.9) and K-ras^{LA1}-mMCP-5$^{-/-}$ (9.2±2.5) mice (mean±SEM, $p<0.05$ for all). This difference relative to K-ras^{LA1} mice persisted at week 14 for MC- and mMCP-5-deficient mice, but not for the mMCP-6-deficient mice. In the fibrosarcoma model, tumor appearance (mean) was significantly delayed in both mMCP-5$^{-/-}$ (16.5 days; $p = 0.003$) and mMCP-6$^{-/-}$ (13.5 days; $p = 0.02$) mice relative to WT mice (12 days). The tumors isolated from mMCP-6$^{-/-}$ mice on day 21 were markedly smaller than those from the control mice.

Conclusion: Our data indicate prominent roles for MCs and two of their proteases in the development of lung adenocarcinomas and the growth of a fibrosarcoma in vivo.

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Interleukin-33 and antigen synergistically amplify cytokine production in mast cells by co-operative interactions among signaling pathways and transcription factors

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The recently described interleukin (IL)-33 accumulates in inflamed tissues of patients with atopic or autoimmune diseases. Because mast cells participate in these diseases and their responses to antigen (Ag) are enhanced by inflammatory factors including pathogenic Toll-like receptor (TLR) ligands, we examined whether IL-33 acted similarly on mast cells. We found that clinically relevant concentrations (≤70 pg/ml) of IL-33 markedly enhanced production of inflammatory cytokines and chemokines in Ag-stimulated rodent bone marrow-derived mast cells and RBL-2H3 cells. The synergy between IL-33 and Ag was remarkable in that substantial cytokine production occurred with concentrations of IL-33 and Ag that by themselves were minimally effective in stimulating mast cells. Under these conditions, mast cells exhibited little degranulation but robust production of tumor necrosis factor-α (TNFα), IL-6, IL-13, and other cytokines and chemokines as determined by ELISAs and multiplex assay systems.

IL-33 acts through its own unique ST2 receptor which, being a member of the IL-1/TLR family of receptors, operates through the adaptor, myeloid differentiation factor 88 (MyD88), and the same downstream signaling pathways as do other members of this family. These pathways were activated by IL-33 in mast cells but, in combination with Ag, substantial synergy was apparent at the level of TNFα activated kinase-1 (TAK1) and then transmitted downstream through the JNK, p38 MAP kinase, AP-1, and NF-κB. However, optimal cytokine production also required activation of the Ca^{2+}/calcineurin/NFAT pathway which was not activated by IL-33. This pathway was activated by Ag or selectively by low concentrations (30 nM) of the Ca^{2+}-mobilizing agent, thapsigargin. Either stimulant, in combination with IL-33, enhanced production of TNFα more than 20-fold. This enhancement was blocked by the calcineurin inhibitor, cyclosporin A. Luciferase reporter assays indicated co-operative interactions between NFAT and AP-1 when cells were stimulated by these combinations of stimulants. These results suggest that IL-33 and Ag together promote a more effective combination of signaling pathways for transcriptional activation of cytokine production. The amplification of cytokine production by IL-33 may thus determine the severity of Ag-dependent allergic and autoimmune diseases and is a further example how mast cells can be "retuned" by exogenous and endogenous factors.

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A novel point mutation of c-Kit inhibits receptor internalization, mast cell survival and development

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Mast cell differentiation, survival and activation are controlled by the membrane tyrosine kinase c-Kit upon interaction with stem cell factor (SCF). We have described a novel point mutation, an A to T transversion at position 2388 of the *c-Kit* gene (at the *Pretty2* allele also known as *m2Btlr*), which results in the substitution of isoleucine 787 by phenylalanine (787F), and have analyzed the consequences of this amino acid substitution for c-Kit signaling and mast cell development. The $Kit^{787F/787F}$ mice carrying the single amino acid exchange of c-Kit lacks both mucosal and connective tissue-type mast cells. In bone marrow derived mast cells, the 787F mutation does not affect SCF binding and c-Kit receptor shedding but strongly impairs SCF-induced cytokine production and apoptosis rescue. Furthermore, the synergistic effect of SCF treatment on FcεRI-mediated mast cell effector functions such as degranulation or cytokine production is impaired. 787F mutation inhibits c-Kit receptor ubiquitination and affects c-Kit internalization thus, modulating the shutting off of the SCF-mediated signaling. Together, these observations demonstrate that I787 is essential in regulating MC development and controls receptor down-regulation.

ORAL ABSTRACT SESSION 3: Eosinophils

13

Use of a novel IgY antibody recognizing 6'-sulfated sialyl Lewis X to identify endogenous lung ligands for Siglec-F on eosinophils

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Background: Siglec-F and Siglec-8 are functional paralog inhibitory receptors on mouse and human eosinophils respectively. Both Siglecs preferentially recognize, either in an immobilized form or when presented as a multivalent polyacrylamide polymer, the ligand NeuAcα2–3(6-*O*-sulfo)Galβ1–4[Fucα1–3]GlcNAc (6'-sulfated sialyl Lewis X or 6'-su-sLe^X), but its natural tissue ligand is unknown. It has previously been shown that lung epithelium selectively bind Siglec-F/8-Ig fusion proteins.

Objective: To characterize endogenous tissue Siglec-F lung glycan ligands.

Methods: Distribution of Siglec-F ligands was studied on mouse and guinea pig lung via histochemistry. Essential reagents for this included a Siglec-F-Ig fusion protein, a novel polyclonal IgY antibody generated by immunizing chickens with a viral-like particle conjugated to 6'-su-sLe^X, and appropriate Ig controls. The sialic acid dependency of binding was explored using sialidase tissue pretreatment. Cross-blocking studies were performed to determine if the Siglec-F-Ig fusion protein and the IgY antibody recognizing 6'-su-sLe^X were binding to the same tissue structures.

Results: Using histochemistry, Siglec-F-Ig fusion protein and the IgY antibody recognizing 6'-su-sLe^X (but not controls) bound selectively, and in the same locations and pattern, to normal mouse and guinea pig lung epithelium. Sialidase pretreatment eliminated binding of both molecules. Siglec-F-Ig fusion protein binding was inhibited by pretreatment of lung tissue sections with IgY antibody recognizing 6'-su-sLe^X, but not with control IgY, further suggesting that the epithelial glycan ligand recognized by Siglec-F-Ig fusion protein contains 6'-su-sLe^X.

Conclusion: Siglec-F ligands containing 6'-su-sLe^X are constitutively and selectively expressed by mouse and guinea pig lung epithelium. Engagement of these glycan ligands by eosinophils accumulating in the airways during inflammatory responses may represent a mechanism for inducing their apoptosis and clearance. Experiments to purify and formally identify these Siglec-F ligands are ongoing.

Abstracts

14

The involvement of lysyl tRNA synthetase in gene regulation

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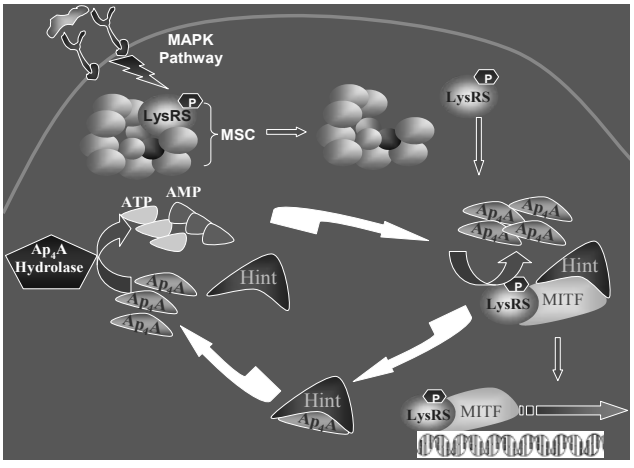
Previously we demonstrated that lysyl tRNA synthetase (LysRS) forms a tertiary complex with either MITF or USF2 and their repressor Hint-1, which is released from the complex by its binding to Ap_4A, enabling MITF and USF2 to transcribe their target genes. We hypothesized that in this complex LysRS is responsible for the synthesis of Ap_4A, which we assumed regulates the transcriptional activity of MITF and USF2. LysRS is a component of the aminoacyl-tRNA multisynthetase complex (MSC) containing eight other aminoacyl-tRNA synthetases and three noncatalytic components.

Our data show that in mast cells following immunological trigger, LysRS is phosphorylated on serine residues, separated from the MSC and that Ap_4A production by this LysRS is significantly increased [1].

Thus, transcriptional activity in this context is regulated by Ap_4A, suggesting that Ap_4A is a second messenger. For Ap_4A to be unambiguously established as a second messenger, several criteria have to be fulfilled, including the presence of a metabolizing enzyme. Since several enzymes are able to hydrolyze Ap_4A, we provided evidence that the nudix-type-2 (nudt2) gene product, Ap_4A hydrolase, is responsible for Ap_4A degradation following immunological activation of mast cells. Knock-down of Ap_4A hydrolase modulated Ap_4A accumulation, resulting in changes in the expression of MITF and USF2 target genes [2].

Recently**,** we have found this pathway exists also in activated cardiomyocytes, and T and B cells. Thus, it seems to us that this newly discovered pathway is triggered in variety cell types. We will discuss in the present workshop our new data regarding the establishing of Ap_4A synthesized by LysRS as a second messenger in the regulation of gene expression (see model below).



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New extracellular structures generated by eosinophils: Role of mitochondrial DNA

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Although eosinophils are considered as being useful in defense mechanisms against parasites, their exact function(s) in innate immunity remains unclear. We have recently obtained evidence for a novel eosinophil-mediated defense mechanism that seems to play a role in the gastrointestinal immune system. We show here that lipopolysaccharide (LPS) from gram-negative bacteria activates interleukin (IL)-5 or interferon (IFN)-γ primed eosinophils to release mitochondrial DNA in a reactive oxygen species (ROS) dependent manner, but independent of eosinophil death. Strikingly, the process of DNA release occurs with high speed in a catapult-like manner in less than 1 second. In the extracellular space, the mitochondrial DNA and the granule proteins form extracellular structures able to bind and kill bacteria both in vitro and under inflammatory conditions in vivo. Moreover, following cecal ligation and puncture, IL-5 transgenic but not wild-type mice demonstrated intestinal eosinophil infiltration and extracellular DNA deposition in association with protection against microbial sepsis. These data suggest a novel mechanism of eosinophil-mediated innate immune responses that might be important to maintain the intestinal barrier function following inflammation-associated epithelial cell damage preventing the host from uncontrolled invasion of bacteria.

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Acting out of the box: Extracellular eosinophil granules are secretory competent cluster bombs

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Human eosinophil granules contain cationic proteins, (e.g., ECP) and other preformed proteins, including diverse cytokines. Mechanisms governing secretion of granule-derived proteins underlie the biologic activities of eosinophils in health and disease. Compound exocytosis, whereby the entire granule contents are released extracellularly following fusion of granules with plasma membranes, occurs when eosinophils interact with large targets, such as helminthic parasites; but otherwise is neither commonly observed *in vivo* nor parsimonious in selectively secreting specific granule-derived cytokines or other proteins. Instead, secretion of granule contents from within intact eosinophils occurs by a process termed, piecemeal degranulation (PMD).

We now investigate an as yet enigmatic, unstudied alternative mechanism by which eosinophil granule-derived proteins might be secreted based on responses of extracellular eosinophil granules. Intact, membrane-bound granules extruded from eosinophils have long been recognized in diverse disorders (e.g., asthma, dermatitis, urticaria and helminth infections). We demonstrate that cell-free eosinophil granules function as independent secretory organelles capable of responding to a cytokine (IFN-gamma), a chemokine

(eotaxin-1), and cysteinyl leukotrienes via cognate membrane-expressed receptors, topologically oriented with ligand-binding domains displayed externally on granule membranes. Granule membrane-expressed receptors, coupled to intragranular signaling cascades, stimulate selective, agonist-elicited secretion of ECP and cytokines from within cell-free eosinophil granules.

These findings have novel cell biology implications: 1) intracellular-derived granules express membrane-bound receptors for cytokines, chemokines and cysteinyl leukotrienes oriented to engage these "extracellular" ligands; 2) these ligand-receptor engagements are coupled to intragranule signaling cascades; and ligand-elicited granule secretion utilizes intragranular membranotubular vesicular secretory mechanisms; 3) cell-free eosinophil granules can differentially secrete their preformed cytokines and other granule proteins. For eosinophils the cytolytic release of intact eosinophil granules yields extracellular organelles fully capable of ligand-elicited active secretory responses able to act as functional "cluster bombs."

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Eosinophils are a major source of transforming growth factor at mucosal sites

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Rationale: Transforming growth factor (TGF)-β plays an important role for the development and function of the mucosal immune system. Impaired TGF-β homeostasis is observed in the pathogenesis of chronic inflammatory diseases such as allergy. However, our knowledge about TGF-β expression in human mucosal tissues is limited. The aim of our study was to analyze TGF-β expression in human intestinal mucosa and to determine major cellular sources such as regulatory T-cells (Tregs) that are also regulated by this growth factor.

Methods: TGF-β expression in human intestinal tissue specimens was analyzed by immunofluorescence double stainings and quantitative RT-PCR. Eosinophils were isolated from peripheral blood and analyzed by means of immunofluorescence, western blot analysis, and RT-PCR.

Results: Surprisingly, eosinophils, and not Tregs, were the most frequent TGF-β positive cells in human gut. Approximately 90 % of TGF-β positive cells were eosinophils. However, only 50 % of eosinophils expressed TGF-β. Levels of TGF-β positive cells varied in different tissues. Measurement of TGF-β mRNA by means of real time RT-PCR confirmed these results. PCR analysis revealed that the relative TGF-β mRNA expression was highest in eosinophils purified from intestinal tissue compared to other intestinal cell fractions or intestinal tissue as a whole. Noteworthy, eosinophils, like Tregs, expressed also the transcription factor FoxP3 suggesting functional similarities between both cell types.

Conclusions: Eosinophils are the main source of TGF-β in human intestinal tissue. Moreover, eosinophils express the Treg marker FoxP3 suggesting that eosinophils have important regulatory functions within the intestinal mucosa that might play a role in intestinal allergy.

ORAL ABSTRACT SESSION 4: Mast Cells and Basophils

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Murine basophils are mature and activated upon arrival in the popliteal lymph node after papain footpad injection

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Rationale: Basophils have recently received attention as antigen presenting cells that drive Th2 development upon exposure to certain allergens and helminths. The aim of this study was to further characterize their recruitment, activation and survival kinetics.

Methods: Papain, a papaya-derived cysteine protease that can cause human Type I hypersensitivity manifested by asthma and anaphylaxis, was injected into footpads of various BALB/c and C57BL/6 mice. At various times, we isolated the cells from bone marrow (BM), spleen and popliteal draining lymph node (DLN), and used flow cytometry to enumerate and characterize the basophils, identified as the FcεRIα+, CD49b+, B220-, CD4-, and c-kit-population. We assessed this population for expression of IL-4, MHC Class II (MHCCLII), CD200R1, and Annexin V.

Results: We verified previous findings that basophils rapidly enter the DLN and peak in number 2.5-3 days post-injection, then rapidly decline over the next 3 days. The response was similar in both the BALB and BL/6 strains with regards to the dose response and to the time course of appearance and disappearance from the DLN. We also evaluated several markers of maturation and activation. Over 90% of basophils in the DLN 2 days after injection expressed IL-4, MHCCLII, and CD200R1, the earliest time point at which this population could be reliably identified. Most basophils in the DLN were weakly positive for Annexin V 2 days after papain injection and this increased in intensity as cell number decreased over the next several days. These cells could also be identified in the spleen and BM yet only the percentage of basophils found in the spleen changed after injection.

Conclusions: The expression of MHCClII, CD200R1, and IL-4 suggests DLN basophils arrive in an activated state, or are activated very rapidly after recruitment to this site from the spleen which acts as a reservoir for the rapid mobilization of these cells to peripheral sites. Their rapid appearance is followed by a rapid disappearance due at least in part to apoptotic cell death based on the high levels of Annexin V expression in these cells 4 days post injection.

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Mast cells in severe asthma: Evidence for alteration in phenotype, location and associated activation

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Background. Mast cells have been implicated in the pathogenesis of asthma, but much remains unknown about their phenotype and activation in relation to increasing severity of asthma. Recent studies have suggested a strong mast cell signature in airway epithelial cells in mild asthma.

Methods. Endobronchial biopsies and lavage were obtained from asthmatic and norrmal subjects (n=120). Severity was defined as previously described (Moore JACI 2007). Additional

subjects (n=43) from U Pittsburgh had epithelial brushings performed. The biopsies were fixed in formalin, embedded in paraffin and immuno- histochemical analysis performed for tryptase and chymase as markers of total mast cells and the MC_{TC} subset respectively. Lavage was analyzed for tryptase (Schwartz/VCU) and prostaglandin (PG) D2. The brushings were analyzed for tryptase and carboxypeptidase A (CPA)3 mRNA by quantitative RT-PCR.

Results. While MC_{Tot} cells in the submucosa peaked in mild asthma in the absence of treatment with corticosteroids (CS), declining with increasing severity of disease, MC_{TC} cells progressively increased with increasing asthma severity leading to a highly significant increase in the ratio of MC_{TC} to MC_{Tot} in severe asthma ($p<0.002$). Similarly, MC_{Tot} were highest in the epithelium in mild asthma/no ICS, but in contrast to submucosa, were not significantly lower in severe asthma. Epithelial MC_{TC} cells were highest in severe asthma. Epithelial brushing CPA3 and tryptase mRNA supported the IHC data. Lavage PGD2 was highest in severe asthma ($p=0.02$), while tryptase levels did not differentiate the groups.

Conclusions. Severe asthma is associated with an increase in the MC_{TC} phenotype which is present in both the submucosa and uniquely in the epithelium. This mast cell phenotype is associated with evidence for activation through increased levels of PGD2 in BAL fluid. These results suggest that approaches which target this altered mast cell population, location and activation may lead to improvements in asthma outcomes.

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Activated T cells release microparticles that activate mast cells

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Background: Close physical proximity between mast cells and T cells has been demonstrated in several T cell mediated inflammatory processes such as rheumatoid arthritis and sarcoidosis. However, the way by which mast cells are activated in these T cell-mediated immune responses has not been fully elucidated. We have previously identified and characterized a novel mast cells activation pathway initiated by physical contact with activated T cells, and showed that this pathway is associated with degranulation and cytokine release. We hypothesized that mast cells may also be activated by microparticles released from activated T cells that are considered as miniature version of a cell (*Nature Rev Immunol 9:581 2009).*

Methods: Microparticles of MW>100,000Da were isolated from supernatants of activated T cells by Centricon filtration or by high-speed centrifugation and identified by EM, flow cytometry (Annexin stain), and expression of integrins. Degranulation and cytokine production by both LAD2 mast cells and human cord blood mast cells were assessed by β-hexosaminidase release and ELISA respectively. Involvement of MAPK signaling pathway in this novel pathway of activation was confirmed by western blotting and confocal microscopy.

Results: The present study demonstrates that stimulated T cells generate microparticles that induce degranulation and cytokine (IL-8 and oncostatin M) release from human mast cells. Confocal microscopy and western blotting demonstrated Ras activation and Erk phosphorylation respectively. The results were similar when mast cells were stimulated by activated fixed T cells or by whole membranes of the latter. This suggests that microparticles carry similar mast cell-activating factors to cells from which they originate.

Conclusions: By releasing microparticles, T cells might convey surface molecules similar to those involved in the activation of mast cells by cellular contact. By extension, microparticles might affect the activity of mast cells, which are usually not in direct contact with T cells at the inflammatory site.

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Mast cells and regulatory T cells in allergic inflammation: Who regulates who and what?

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Chronic allergic inflammation, e.g. in patients with atopic dermatitis, is associated with increased numbers and activation of mast cells and decreased numbers and function of regulatory T cells (T regs). These changes may occur independently or because both cell populations control and suppress each other. Here, we have investigated and characterized the effects of mast cells on T regs (numbers and functions) and the effects of T regs on mast cells. Interestingly, we found that T regs, but not T effector cells, cocultered with mast cells show a dose-dependent reduction of numbers, CD25 expression, and function. Along the same line, T regs obtained from mast cell-deficient Kit^{W}/Kit^{W-v} mice were increased in numbers, exhibited higher rates of proliferation, and elevated IL-10 production as compared to T regs from wild type *Kit*+/+ mice. For example, Kit^{W}/Kit^{W-v}-derived T regs showed a proliferation rate of 12% and release of 227 pg/ml IL-10 as compared to 3.6% proliferation ($p<0.001$) and 30 pg/ml IL-10 release ($p=0.006$) in *Kit*+/+-derived T regs. These findings suggest that mast cells can control and suppress T reg populations in terms of numbers and functions. On the other hand, murine peritoneal mast cells showed pronounced and rapid degranulation after coculture with T regs, but not T effector cells. Also, T regs, but not T effector cells, induced the release of LTB4 and proinflammatory cytokines such as IL-1 and IL-6 in cocultered mast cells (e.g. IL-6: 4.236 pg/ml as 3pg/ml in controls, $p<0.005$). Furthermore, T regs significantly upregulated mast cell proliferation. These data indicate that T regs can activate mast cells and increase their numbers, whereas mast cells downregulate T reg numbers and functions. The effects of mast cells and T regs on each other may contribute to the development of allergic reactions and may be novel targets of better therapeutic strategies for allergy treatment.

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Infections affect mast cell reactivity – possible implications for asthma exacerbations

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The IgE-dependent activation of mast cells is well-known and has been described extensively during the last century. During the last decades it has also been evident that mast cells express pattern recognition receptors and can recognize and be activated by pathogens through binding via e.g., Toll-like receptors (TLRs), C-type lectin receptors (CLRs) or NOD-like receptors (NLRs).

However, few studies have addressed the question how pathogens/infections affect the IgE-dependent mast cell activation. Since airways infections of viral or bacterial origin are a common cause of asthmatic exacerbations we decided to investigate how *in vitro* "infection" affected IgE-dependent mast cell reactivity. For the study we used murine bone marrow cells that were differentiated into either connective tissue like mast cells (CTLMC) or mucosal like mast cells (MLMC). Real-Time PCR array was used to measure expression of TLRs, and inflammatory mediators in MLMC and CTLMC. Mast cells were cultured in the presence of TLR ligands for short (24 h) or long term (96 h) and the effect on IgE-mediated release of inflammatory mediators was measured. We found a differential expression of TLRs on CTLMC and MLMC, respectively, with higher expression of TLR-1, 2, 3, 5, 7, 8, and 9 in CTLMCs, while TLR-4 and 6 were equally expressed in CTLMC and MLMC. When mast cells were cultured in the presence of TLR-ligands and then activated with IgE+antigen, we found an increase in the IgE-mediated release of mediators, i.e., through degranulation, eicosanoid synthesis, and cytokine secretion. Thus, our data suggest that prolonged exposure of mast cells to pathogens/TLR-ligands affects their reactivity by priming them for increased release of inflammatory mediators when they are activated by IgE-receptor aggregation. Whether this has any impact on asthma exacerbations need to be investigated further in animal models and eventually in humans.

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Vitamin D_3 promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice

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Mast cells are key players in inflammation and tissue remodeling in IgE-dependent allergic disorders, as well as in certain innate and adaptive immune responses that are thought to be independent of IgE. Importantly, the historical view that mast cells contribute primarily as pro-inflammatory effectors of immune responses has been challenged; there is now evidence that mast cells can also play an important negative regulatory role to re-establish tissue homeostasis following an inflammatory response. We reported that mast cells and mast cell-IL-10 can limit the skin pathology associated with chronic low-dose ultraviolet (UV)-B irradiation (M. A. Grimbaldeston et al. *Nature Immunology*, 2007). We now demonstrate that activation of mast cells via the receptor for vitamin D_3 (VDR) can promote mast cell IL-10 production and the reduction of UVB-induced skin pathology.

Exposure of the skin to UVB irradiation induces alterations in the cutaneous microenvironment at affected sites, including the production of the immune modifying agent 1α,25-dihydoxyvitamin D_3 (1α,25$(OH)_2D_3$). We found that 1α,25$(OH)_2D_3$ can upregulate IL-10 mRNA expression and induce IL-10 secretion in mouse mast cells *in vitro*. To investigate the roles of 1α,25$(OH)_2D_3$ and mast cell VDR expression in chronically UVB-irradiated skin *in vivo*, we selectively engrafted the skin of genetically mast cell-deficient WBB6F_1-*Kit*$^{W/W\text{-}v}$ mice with bone marrow-derived cultured mast cells obtained from C57BL/6 wild-type or *VDR*$^{-/-}$ mice. We found that optimal mast cell-dependent suppression of the inflammation, local production of pro-inflammatory cytokines, epidermal hyperplasia and epidermal ulceration associated with chronic UVB irradiation of the skin in *Kit*$^{W/W\text{-}v}$ mice required expression of VDR by the adoptively-transferred mast cells. Our findings strongly suggest that 1α,25$(OH)_2D_3$/VDR-dependent induction of IL-10 production by cutaneous mast cells contributes substantially to the mast cell's ability to suppress inflammation and skin pathology at sites of chronic UVB irradiation.

POSTER SESSION 1: Genetic and Environmental Factors in Allergic Disorders

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New high-throughput strategies for systemic phenotype analysis of mutant mouse lines as models for IgE-mediated allergic diseases

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With the completion of human and mouse genome sequences, more than 30,000 mutations in ES-cells will be engineered and thousands of mouse disease models will become available, thus raising the potential of mouse models for IgE-mediated allergic diseases to establish a link between genetic predisposition and allergic phenotype. To identify phenotypic alterations in mice, a systemic allergy screening platform able to detect new phenotypes in large groups of animals - both under baseline and/or challenge conditions – using limited amount of biologic sample is required. The aim of this study was to establish such high-throughput technologies for detecting IgE-mediated allergy phenotypes within the German Mouse Clinic (GMC), a large scale comprehensive phenotyping center for mutant mouse lines (MML).

MML showing an interesting primary phenotype (e.g., change in total plasma IgE) are subjected to a more in-depth assessment. This includes a model of allergic sensitization and aerosol challenge with different optional allergens (OVA, Phl p 5 or Api m 1-10). Immunoglobulins are rapidly monitored from a single plasma sample using a Luminex bead-array technology. Cells (Eos, PMN, MΦ, T, B and NK cells) from bronchoalveolar lavage (BAL) and immune phenotyping of lymphocytes are each analyzed by one-step, single staining multi-color flow-cytometry. Additionally, a single-step quantification of multiple cytokines from BAL fluid is performed. Gene expression profiling, detailed histology and lung function can be offered through the GMC center (www.mouseclinic.de).

The equivalence and validity of the bead-array technology for the detection of immunoglobulins was established. In cellular BAL analyses, flow cytometric determination correlated significantly with the differential morphological count using cytospins, but consuming less time and manpower. All methods proofed to be highly useful for the high-throughput phenotypic allergy analysis in large cohorts of animals. The phenotyping methods were also successfully applied in a murine model of SIT using various MML.

In conclusion, we have successfully established a systemic phenotypic allergy screening platform to identify distinct gene functions in MML that enhance or reduce allergic disease in the murine model. These high-throughput technologies are likely to provide important advances with regard to pathophysiology, diagnosis, and therapy of allergic diseases. (support: BMBF-NGFNplus-01GS0868)

Abstracts

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Polymorphisms in Claudin-1 (CLDN1) and risk of asthma in independent populations of African descent

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Rationale: Human claudin-1 gene (*CLDN1*) is one of 21 members of a gene family that encode highly related proteins critical in tight junction formation and function. *CLDN1* variants have recently been implicated in hepatitis C virus infection, cancer and atopic dermatitis. We tested the association between single nucleotide polymorphisms (SNPs) in *CLDN1* and asthma phenotypes in two independent populations.

Methods: The ABI 7900 System and the Illumina GoldenGate platform were utilized to genotype 17 tagging SNPs in *CLDN1* in 464 African American asthmatics and 471 non-asthmatic controls and a replicate African Caribbean population of 153 asthmatics and nuclear and extended family members from Barbados. Tests for association with asthma were performed using Generalized Estimating Equations (GEE) method in the African American population and MQLS (V1.5.5) in the African Caribbean families. Association with total serum IgE (tIgE) concentrations was tested utilizing GEE method in both populations.

Results: Two intronic SNPs (rs893051 and rs3774032) were significantly associated with asthma in the African American group ($P = 0.011$ and 0.017, respectively). Among the Barbados asthmatic families, a trend towards association was also observed between asthma and two additional SNPs (rs11717803 and rs9290927) in intron 1 and downstream of the gene ($P = 0.044$ and 0.043, respectively). Replication was found for one SNP rs893051 significantly associated with tIgE ($P = 0.012$), which was the same marker associated with asthma in the African Americans.

Conclusions: Our findings implicate a novel genetic contribution of *CLDN1* polymorphisms to asthma.

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Measuring allergens and pollen across Europe: The EU-project HIALINE

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Exposure to allergens is one of several factors determining sensitization and allergic symptoms in individuals. Exposure to aeroallergens from pollen is assessed by counting allergenic pollen in ambient air. However, proof is lacking that pollen count is representative for allergen exposure. We therefore monitored simultaneously birch, grass and olive pollen counts and their corresponding major pollen allergens Bet v 1, Phl p 5 and Ole e 1 across Europe.

Already at one location in Europe in Munich, Germany, we found that the same amount of pollen from different years, different trees and even different days released up to 10-fold different amounts of Bet v 1. Thus exposure to allergen is poorly monitored by only monitoring pollen count. Monitoring the allergen itself in ambient air might be an improvement in allergen exposure assessment.

The objective of the HIALINE-project (Health effects of airborne allergen information network) is to evaluate if these effect found in Munich, Germany are also measurable over a bigger geographic area like Europe, and at the same time implement an outdoor allergen early warning network, in addition to the pollen forecasts. Climatic factors that influence allergen exposure will be extracted and will be used to calculate the effect of climate change on local airborne allergen exposure.

The major allergens from the top 3 airborne allergens in Europe (grasses, birch and olive) are sampled with a cascade impactor, extracted and analyzed by allergen specific ELISA´s. Pollen counts are measured by standard pollen traps and correlated with the weather data. Allergen forecast will be calculated by incorporating the SILAM chemical transport model and compared with the observations of HIALINE aiming at a comprehensive parameterization of the allergen release and transport.

Expected outcomes are the implementation of a network of European outdoor allergen measurements to better predict allergic symptoms. Also the climatic factors that govern allergen exposure in outdoor air will be established. These can be used to calculate the effect of climate change on the health effects of airborne allergens.

The Project consists of 13 partners in 11 countries and is financed for 3 years

Acknowledgement: The research leading to these results has received funding from the Executive Agency for Health and Consumers (EAHC) under grant agreement No 2008 11 07

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Molecular and genetic studies around the relationship between ascariasis and asthma in humans

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Background Allergy and helminth infections have evolutionary links and influence each other in pathogenesis and prevalence; however, some aspects, like the clinical impact of IgE cross reactivity between mites (such as *D. pteronyssinus* and *B. tropicalis*) and Ascaris antigens have not been analyzed.

Objective To evaluate the genetic and molecular specificity of the IgE responses to Ascaris and mite antigens in asthmatics and healthy subjects living in the tropics

Methods The investigations were approved by the Ethics Committee of the University of Cartagena. Cross reactivity studies were done using ELISA and immunoblotting inhibition assays with extracts and recombinant allergens. Cross reacting allergens were confirmed by mass spectrometry. Genetic studies on the antibody response to Ascaris and mite allergens included 1064 subjects in case control design. Polymorphisms of three candidate genes at 13q33 were genotyped using TaqMan assays. Linear and logistic regressions were used to model effects of genotypes on antibody levels.

Results A weak association between IgE to the Ascaris extract and asthma disappeared when adjusting for IgE to mites. No association was detected when using IgE to ABA-1 as a marker for infection. We demonstrated for the first time that there is a high degree of cross reactivity (70% inhibition) between Ascaris and mite extracts; at least four Ascaris allergens were involved, among them tropomyosin and glutathione-S-transferase. There was no cross reactivity between ABA-1 and any component of mite extracts. The GG genotype of *LIG4* was associated with higher IgE levels to Ascaris ($p = 0.008$). The GG genotype of *BAFF* was associated with higher IgG levels to Ascaris ($p = 0.001$) and IgE to ABA-1 ($p = 0.01$). There was no association with asthma or mite sensitization.

Conclusions In addition to the potential implications on the pathogenesis of asthma and ascariasis, cross reactivity between mite and Ascaris allergens could bias individual diagnosis and epidemiologic surveys. The use of ABA-1 as a more specific antigen from Ascaris is recommended. Even though the existence of cross reactivity we found that genes protecting against parasite infections can be different to those predisposing to asthma and atopy. Funded by Colciencias

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How immune peptidases change specificity: Cathepsin G gained tryptic function by missense mutation during primate evolution

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Introduction: Cathepsin G is a secreted serine peptidase expressed highly by neutrophils and mast cells and to lesser extents by macrophages and dendritic cells. Studies in *Ctsg*-null mice suggest that cathepsin G supports antimicrobial defenses but can injure host tissues. The human enzyme has unusual "Janus-faced" ability to cleave peptides at basic (tryptic) as well as aromatic (chymotryptic) sites, with a variety of observations suggesting that tryptic activity underlies important functions such as activation of pro-urokinase plasminogen activator. Crystallographic studies attribute tryptic activity to acidic Glu^{226} in the primary specificity pocket. However, most mammals, including mice, contain Ala^{226} rather than Glu^{226}.

Methods: To test the hypothesis that tryptic activity in humans is anomalous, human cathepsin G was compared with recombinant mouse wild type, macaque/gorilla/chimpanzee-like $Ala^{226}Glu$ and human-like $Ser^{189}Ala/Ala^{226}Glu$ mutants of mouse cathepsin G.

Results: These studies reveal that mouse cathepsin G differs from the human enzyme in lacking tryptic activity and resisting tryptic peptidase inhibitors (e.g., aprotinin), while being more active in hydrolyzing chymotryptic substrates and favoring angiotensin destruction over activation. $Ala^{226}Glu$ mutants of mouse cathepsin G acquire tryptic activity and human ability to activate pro-urokinase. Phylogenetic analysis reveals that the $Ala^{226}Glu$ transformation, which occurred in primates 31-43 million years ago via missense mutation, represented a non-classical and apparently unprecedented means of creating a tryptic peptidase.

Conclusions: The tryptic activity of human cathepsin G is neither typical of mammalian cathepsin G nor an attribute of the ancestral enzyme, which was primarily chymotryptic. More generally, broadening of specificity in the primate lineage opposed the general mammalian trend of increased specialization by immune peptidases and allowed acquisition of new functions.

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House dust mites studies in Lithuania

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House dust mite fauna in Lithuania consist of 49 species, belonging to 4 Orders (Astigmata, Oribatida, Prostigmata, Mesostigmata) and 20 families. The most abundant were Astigmata mites: 81% of all mites belongs to Pyroglyphidae family. *Dermatophagoides pteronyssinus* (41.2%) and *D. farinae* (15.1%), *Acarus siro* (Acaridae) (5.6%), *Glycyphagus domesticus* (Glycyphagidae) (3.4%) and *Cheyletus eruditus* (Cheyletidae, Prostigmata) (6.7%) were species, predominant in house dust samples.20 mite species were found for the first time in house dust in Lithuania. Pyroglyphidae family: *Dermatophagoides evansi* Fain, Hughes et Johnston, 1967 Acaridae: *Acotyledon sp.*, *Schwiebea tshernyshevi* Oudemans, 1916, *Traupeauia sp* and *Rhizoglyphus echinopus* (Fumouze et Robin, 1868)Glycyfagidae: *Ctenoglyphus plumiger* (C.L. Koch, 1835), *Carpoglyphus lactis* L., 1758 and *Xenoryctes krameri* Michael, 1896 Saproglyphidae: *Calvolia tuberculata* Zachvatkin, 1941 Also 7 Oribatida species, 2 predatory Prostigmata and 2 Gamasina (Mesostigmata) species. 65.23% of identified mites were dermatophagous, 15.57% mites feeding on skin flacks and plant parts, phytophagous- saprophagous were 16.25% and predatory mites found only 3.54%. Mites of the first feeding group predominated in dwellings, pre-schools, hospitals and passenger trains. In libraries and museums 55.86% of all mites were phytophagous-saprophagous. According to our study in children pre-schools more mites were present in mattresses (54.1%) and stuffed toys (80%) of week-stay pre-schools, in the dust of premises of ground floor than the first floor. 15 mite species were found in sanatorium and hospitals, 94.74% of them - representatives of Pyroglyphidae family. In libraries and museums mite fauna is more diverse but 42% of all mites were Pyroglyphidae. For the first time mites fauna was examined in passenger aircraft and trains. Two of seven identified species (*D. pteronyssinus, D. farinae)* were predominant (33.33%) in dust samples gathered from the aircraft seats surface. In the dust of passenger trains mattresses 8 mite species were found. All of them are of an allergenic importance.*D. farinae* mites can spread with the dust from other countries by passenger vehicles, it is obvious because the frequency of these mites number found in house dust during last 18 years increased from 2.13% till 29.83%. House dust mites are important source of allergic diseases. 17.31% of patients in our country are sensitive to house dust, 25.75% show sensitivity to house dust mites. For the first time hypersensitiveness to storage mites were performed. 66.66% of patients, sensitive to *D. pteronyssinus* mites show sensitivity to *Glycyphagus domesticus,* and 23.1% to *Acarus siro* mite allergens. In the blood of allergic patients more IgE were found to *Dermatophagoides pteronyssinus* (Dpt) mites allergens when to *D. farinae* (Df).

Our investigation shows that house dust mites fauna in Lithuania is abundant and widely spread in dwellings and public houses. It is of great vitality and very important for the diagnostic of allergic diseases.

Abstracts

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Gene-environment interactions in utero: Gluthathione-S-transferase polymorphisms, prenatal exposure to acetaminophen and tobacco smoke and risk of childhood asthma

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Background: Prenatal exposure to both acetaminophen and tobacco smoke have been associated with increased risk of asthma in childhood. Demonstration of biologically plausible interactions between these exposures and antioxidant gene polymorphisms of maternal genes would strengthen causal inference.

Methods: Women enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) reported their smoking habits and acetaminophen use during pregnancy. Their children were followed up from birth. Asthma status was established at age 7½ years from parental report. Maternal and child DNA was genotyped for copy number variations of GST M1 and T1 and a single nucleotide polymorphism of GST P1 (Ile105Val, rs1695). Effects of prenatal acetaminophen and tobacco smoke exposure on asthma phenotypes were stratified by maternal and child genotype.

Results: The effect of late gestation prenatal acetaminophen exposure on asthma risk was modified by maternal GSTT1 genotype (P interaction 0.013) and on wheezing risk by GSTT1 and GSTM1 genotype (P interaction 0.041 and 0.035, respectively). The risks were greater when these genes were present (especially two copies), than when they were deleted. The risk of asthma and wheezing associated with maternal GSTM1 genotype was further increased when the gene was also present in the child. There was no strong evidence of effect modification by maternal GST genotypes on the associations between prenatal smoking and asthma or lung function in children.

Conclusions: We have found evidence to suggest that maternal GSTM1 and GSTT1 polymorphisms modify the effect of prenatal acetaminophen exposure on childhood asthma phenotypes, providing evidence for a casual relationship between prenatal acetaminophen and childhood asthma.

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The NPS-NPSR1 pathway in asthma and allergy: An update 2010

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The NPSR1 gene (earlier known as GPRA or GPR154, Laitinen & al. Science 304:300, 2004) was implicated in asthma susceptibility first in Finnish and Canadian founder populations. Follow-up work by others and us has abundantly replicated its association to asthma in several European, American and Chinese populations, with few negative reports. NPSR1 has been convincingly excluded as a susceptibility gene for atopic eczema, but association studies suggest that NPSR1 might play a role in inflammatory bowel disease and respiratory distress syndrome of the newborn. Gene-environment interaction studies have suggested that NPSR1 polymorphisms modify the protective effect of early farm animal exposure on asthma risk, and functional study of NPSR1 has suggested that this effect might be mediated at least in part by macrophages. Cell models used to identify downstream target genes of NPS-NPSR1 signalling have revealed that the alpha chain of glycoprotein hormones (CGA) is the most strongly regulated target gene, with additional genes of relevance to asthma. Recently, the different signalling properties of the NPSR1-A and –B isoforms have been elucidated. Overall, the A isoform is a more potent signalling receptor, with B isoform only showing stronger induction of CD69. Functional study of the NPSR1 promoter have revealed that it contains several functionally relevant polymorphic elements that can modulate the expression level of NPSR1. Most recently, detailed study of NPS and NPSR1 expression in the gut revealed that enteroendocrine cells express relatively high levels of the ligand and receptor, and that stimulation with NPS resulted in a dose-dependent upregulation of CGA, tachykinin 1 (TAC1), neurotensin (NTS) and galanin (GAL), all encoding peptide hormones secreted by enteroendocrine cells. These results have revealed a possible role for the NPS-NPSR1 pathway in cells of neuroendocrine origin, and suggest that further studies of such cells in the airways are highly warranted.

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Expression of innate immune receptors in cord blood leucocytes and atopic dermatitis during the first two years of life

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Cross-sectional studies investigating children growing up on a farm at school age have shown that environmental exposures may alter the children's innate immune system and confer protection against the development of respiratory allergies; early life or even prenatal exposure was most effective. No clear effect of the farming environment on the development of atopic dermatitis could be made evident in these studies.

The PASTURE study is a prospective birth cohort study involving children from rural areas in Austria, Finland, France, Germany and Switzerland. Women were recruited during the third trimester of pregnancy and the children followed-up after birth. We investigated whether immunological effects of prenatal exposures might be reflected by alterations of innate immune receptors in cord blood and how such possible changes would correlate with AD.

From 961 children from the PASTURE birth cohort data were available on doctor's diagnosis of atopic dermatitis between the age of 1 and 2 years. In 818 of these children gene expression of Toll-like receptors 1 to 9 and CD14 in cord blood leukocytes was analysed by quantitative PCR. TLR3 gene expression was below detection limit in most samples and not entered into analysis. Based on the number of TLRs in the lowest tertile, a TLR expression score ranging from 0 to 9 was established. In 29% of the children with the lowest score a doctor's diagnosis of atopic dermatitis had been documented, as opposed to 10.4% of the children with the highest score. Statistical regression analysis showed a decrease in the risk for atopic dermatitis of 0.09 for each step of increase in the score (p=0.005).

We conclude that the higher the expression of innate immune receptor genes was at birth, the lower was the risk for developing

atopic dermatitis during the first two years of life. We propose that alterations of the innate immune system evident already at birth may reflect intrauterine exposures, and that intrauterine gene-environment interactions may determine development of atopic dermatitis during the first years of life.

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Genotype determined expression of genes differentially expressed in acute childhood asthma

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Introduction: We have used a microarray analysis to identify genes differentially expressed in acute childhood asthma. Peripheral blood mononuclear cells (PBMC) were examined from children presenting to an emergency department with acute asthma (Ac) and followed-up in convalescence (Cv). We identified 52 genes that were significantly differentially expressed. In these genes, we have previously identified polymorphisms in 4 genes related to the absolute levels of mRNA found in PBMC during the acute episode. In the current analysis, we have investigated possible relationships between the differential levels of mRNA (ie the acute minus the convalescent level) and SNPs for the same 52 genes.

Hypothesis: SNPs in the genes identified by micro-array alter differential expression levels of the encoded product during acute asthma.

Methods: Fifty children aged 2-15 years with acute asthma were recruited on presentation to hospital. mRNA levels of 21 candidate genes were measured using qRT-PCR of mRNA extracted from PBMCs collected at acute attack. For these genes, 87 SNPs were genotyped by the Australian Genome Research Facility. Logged differential expression levels were compared between genotypes using multiple regression, adjusted for age, sex and time since steroids were administered.

Results: Mean age of the children was 7.0 years, 58% male, 88.6% atopic and infected with a respiratory virus (84.2%). Of the 21 genes, two SNPs had genotypes with significantly higher acute mRNA levels than other genotypes.

Table: mRNA differential expression level (acute minus convalescent levels) for genotypes of SNPs in candidate genes identified by micro-array

Gene	SNP	Genotype	GM mRNA differential	p
THBD	rs1042580 A/G	AA	8.20	p = 0.030
		AG	8.05	
		GG	7.31	
MX1	rs464138 A/C	AA	10.2	p = 0.022
		AC	10.1	
		CC	7.5	

Conclusion: We have identified two genes with the greatest degree of genetically-determined differential upregulation of expression during acute asthma. These genetic variants may alter inflammatory mediator expression in acute asthma and are likely to contribute to attack severity and provide a target for specific therapies.

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CD14 polymorphisms and serum CD14 levels through childhood; a role for gene methylation?

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Background: CD14 is a pattern recognition receptor for environmental lipopolysaccharide (LPS), and engagement of the CD14-LPS complex activates innate host defence mechanisms. Single nucleotide polymorphisms (SNPs) in the *CD14* gene have been associated with soluble CD14 (sCD14) levels, but inconsistencies between studies suggest the presence of regulatory mechanisms hitherto not well understood.

Objective: To investigate possible associations between *CD14* SNPs and sCD14 levels at different time points in childhood (at birth (cord blood), two and 10 years), and to explore whether these associations were related to *CD14* gene methylation.

Methods: Four SNPs, rs2569191 (-1145GA), rs5744455 (-550CT or -651CT), rs2569190 (-159CT or -260CT), and rs4914 in *CD14* were genotyped in 762 children from the Environmental and Childhood Asthma study. Genotype frequencies were analysed for association with sCD14 levels in 660 babies, 346 children at age two and 360 children at age ten. In a subgroup of 157 children with DNA available at both two and 10 years of age, *CD14* methylation patterns were determined and analyzed against detected *CD14* gene-sCD14 associations.

Results: rs2569191, rs5744455 and rs2569190 were associated with sCD14 levels at birth and at two years, only rs5744455 was associated with sCD14 levels at 10 years. *CD14* methylation increased significantly from age two to 10 years, and level of methylation was inversely correlated with sCD14 levels at 10 years.

Conclusion: The reduced impact of *CD14* polymorphisms on sCD14 levels from early to late childhood paralleled a small but significant increase in *CD14* methylation during the same period.

"**Clinical implications"**

The epigenetic changes indicated by gene methylation imply gene-environment interactions in early life that may explain reported inconsistencies in the role of CD14 polymorphisms and risk of atopic disease in childhood.

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Epidemiological study of specific IgE responses among junior high school students in Wakayama, Japan by using a solid phase multiplex technology

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It was reported in the previous CIA symposium that a solid phase multiplex technology named Optigen was useful to evaluate specific IgE responses to whole milk extracts and allergenic proteins simultaneously. This method is able to measure the amounts of specific IgE in 200uL of serum for each of 33 different allergens and is likely to be also available for epidemiological studies of allergic disorders. Thus we have conducted a study to investigate the positive rates of specific IgE antibodies among the students in Wakayama

prefecture in Japan by using sera collected in two separate occasions in the same junior high school.

The sera drawn from 312 and 649 junior high school students each in 1995 and 2002 were used for this study. A mixture of 200uL of subject serum and 400uL of diluent were incubated in a custom-designed reaction reservoir called a pette body, followed by reaction with HRP-conjugated anti-human IgE. After the addition of photoreagents, the levels of luminescence generated were determined by using a luminometer.

The results showed that the positive rates of specific IgE antibodies against inhalant allergens such as house dust mite, cat epithelium, Japanese cedar and cypress pollens increased dramatically, while those against food allergens did not change so much. It was also shown that the average number of positive allergens per sample increased from 2.5 to 3.2 and that this increase was more prominent in students suffering from allergic rhinitis. It was concluded that specific IgE responses among junior high school students were enhanced very much during these seven years.

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Immunologic and environmental modulation of the "Allergic March"

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We and others have previously shown that allergic rhinitis is an important risk factor for the development of asthma, allergic rhinitis patients being more likely to report new onset asthma in multivariate models with OR ranging from 3.2 to 7.8.

Little is known about the factors modulating this progression. Considering the well-known ability of allergen immunotherapy to induce immune-tolerance, it has been hypothesized that early immunotherapy may reduce progression to asthma in allergic individuals. We and others have shown that treatment with allergen immunotherapy lowers the risk of the development of new asthma cases in patients with allergic rhinitis by as much as a 50%.

Conversely, in view of the known ability of cigarette smoke to potentiate allergic immune responses and enhance allergic inflammation, it is anticipated that cigarette smoking is an important environmental factor that may accelerate the progression of rhinitis to asthma. We have recently demonstrated that smoking is strongly related to the risk of incident asthma in allergic rhinitic patients with a clear dose-response association; in the multivariate analyses, those smoking 1-10 pack-years had an OR 2.14, those with 11-20 pack years had an OR 3.81, and those smoking more than 20 pack years had and OR 5.87 compared to never smokers.

The notion that early treatment of allergic individuals with immunotherapy can reduce progression to asthma and that iindividuals with allergic rhinitis who smoke are at higher risk of developing asthma, provides evidence of strong immunologic and environmental modulation of the "allergic march".

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Allergen sensitization and total IgE levels during childhood differently associated with perinatal exposure to marine pollutants

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The prevalence of allergy in the arctic regions has generally been assumed to be low, but sensitization has been dramatically increasing during the last decades*. Exposures to marine contaminants are of high concern to populations that rely on seafood for their livelihood, especially when marine mammals are part of traditional diets, as is the case in Inuit communities and the Faroe Islands. The main contaminants are methylmercury and polychlorinated biphenyls (PCBs), while other industrial pollutants, such as dioxins are not similarly increased in the food chains. We have carried out a prospective study of a birth cohort in the Faroe Islands, a fishing community with increased exposures to methylmercury and PCBs from traditional diets that include pilot whale meat and blubber.

Informed consent was obtained from a total of 645 mothers in connection with consecutive spontaneous singleton births at term. The present report is based on the 566 (88%) cohort members, who at 7 years of age underwent examinations for total IgE and specific IgE to grass determined by the ImmunoCAP from Phadia. PCB exposure was determined from analyses of serum, and methylmercury exposure from mercury analyses of whole blood and maternal hair. Maternal serum was obtained in the 34th week of pregnancy. Cord blood and maternal hair for mercury analysis were obtained in connection with the parturition. Serum, whole blood, and hair were taken from the child at 5 and 7 years.

For total IgE concentrations associations ($p<0.01$) were seen with the PCB concentrations at age 5 and 7 years, and a similar tendency for prenatal methylmercury exposure. Adjustment for cofactors in regression analysis did not change the statistical significance of these correlations. Maternal fish intake during pregnancy was not associated with the child's IgE. Conversely, specific IgE concentration showed lower prenatal methylmercury exposure in children sensitized to grass. Although concentrations below 0.35 kUA/L may not be clinically meaningful, a negative correlation was found ($r_s = -0.17$; $p < 0.001$). Again, adjustment for confounders did not affect the significant association with prenatal mercury exposure.

* Krause T, Koch A, Friborg J, Poulsen LK, Kristensen B, Melbye M. The Lancet 2002; 360:691-2

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Evaluation of Immunoglobulin G antibodies against *Aspergillus versicolor* in a group of elderly women as biomarker of mould exposure and surrogate marker of indoor situation

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Mould exposure is perceived as a potential risk factor for development of asthma and allergy as well as other negative health effects like infections, eye and skin irritation and hypersensitivity. Moisture-damaged buildings are often the cause of fungal indoor sources and Aspergillus is one of

the most common fungal detected there. The presence of mould-specific IgG antibodies has been used as biomarkers for mould exposure in several studies. The aim of this study was to evaluate the prevalence of *Aspergillus versicolor* IgG antibodies in a group of 350 women aged 69-79 years (SALIA cohort) who lived for more than 20 years at the same residential address. We further compared the data with reported respiratory health problems, domestic circumstances and with results from the analysis of exhaled breath condensate (EBC) and induced sputum (IS) mediator profile in addition to a current physical examination. Sera were tested for specific IgE to sx1 (inhalative atopy screening test), total IgE and *A. versicolor*-specific IgG (sIgG via ELISA). 14.6% of the sera were positive in the sx1-test and 13.4% had total IgE levels higher than 100 kU/L. In 11.4% of the sera the sIgG concentrations were above 20 mg_A/L. 12.7% of the women reported that they live in a mouldy flat and 4.7% in buildings with dampness, but without correlation to the sIgG response. In IS samples of women with elevated *A. versicolor* IgG concentrations, the TNF-α concentrations was significantly higher and also the IL-8 and IL-1β concentrations showed a tendency to be higher than in IS of women with lower sIgG. Further IS and EBC parameters showed no difference. IS of women who reported "living in a mouldy flat" had significantly higher percentage of neutrophils and a tendency of higher total cell count and TNF-α concentration. Our results demonstrate that the *A. versicolor* IgG concentrations in elderly women do not correlate with the reported information concerning mould contamination and dampness in their flats, even though the increase of several inflammatory markers of the lower airways in these women indicated an adverse health effect and an association between indoor mould exposure and immunological biomarkers.

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The transgenerational asthma-preventive effect of *Acinetobacter lwoffii F78* is mediated by innate and adaptive immune mechanisms together with epigenetic regulation

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Epidemiological studies conducted in the traditional farming environment identified an association between the natural exposure to the gram-negative non-pathogenic microbe *Acinetobacter lwoffii* and the protection of bronchial asthma. Further studies revealed that the pre- and postnatal environment represents an important window of opportunity for allergy prevention. A well-established animal model of experimental asthma was employed to investigate a cause-effect relationship between exposure to *A. lwoffii* and to examine mechanisms of asthma protection. Intranasal application of *A. lwoffii* beginning just before pregnancy and continuing throughout pregnancy had a profound protective effect on the development of experimental asthma in the next generation. The protected phenotype includes eosinophilic airway inflammation, mucus production, development of airway hyperresponsiveness and TH2 inflammation. Utility of quintuple TRL2, 3, 4, 7, 9 -/- mice further showed that this protective effect depended on intact TLR-signalling in the mother. These signalling events resulted in a low-grade inflammatory response, characterized by local and systemic IL-6 increases in the absence of neutrophilic inflammation throughout the exposure period. Upregulation of TLR-receptors in the airways was associated with a markedly reduced expression of TLR-receptors in placental tissues. Further experiments showed that this protective effect was mediated via IFN-γ increases in the offspring, since blockade of IFN-γ using neutralizing monoclonal antibodies prevented the *A. lwoffii* mediated effect. Further analysis concentrated on the IFN-γ promoter. No changes in promoter methylation was found using pyrosequencing. However, histone H4AC acetylation was significantly increased in offsprings from *A. lwoffii* exposed mothers. This increase was accompanied by higher levels of IFN-γ mRNA as well as protein production. That promoter acetylation was indeed responsible for these effects was shown using Garcinol as an inhibitor of H4 acetylation. Garcinol treatment of offspring mice again prevented the *A. lwoffii* meditated effect of asthma protection. These data indicate that transgenerational asthma protection by *A. lwoffii* depends on an intimate interaction between innate and adaptive immunity of the mother and the fetus. The resulting change in TH1/TH2 immunity is operating on the level of epigenetic regulation. These studies provide, therefore, a novel explanation for the epidemiologically observed "farming effect".

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The Role of CD23 in IgE dependent signaling and pharmacotherapy of allergic disease

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CD23 is the low affinity receptor for IgE. It is widely expressed on a variety of cells important to the immune response in allergic disease, and plays a critical role in the regulation of IgE synthesis. The expression of CD23 can be found on cells such as monocytes, macrophages, B cells, eosinophils, dendritic cells, intestinal epithelial cells and airway smooth muscle cells. The spectrum of expression suggests an important role for this molecule in the response to allergens whether they be inhalant or food allergens. The association of genetic variants of CD23 has been well established, and a potential mechanism involving a single nucleotide polymorphism (SNP) that codes for an amino acid exchange at R62W within the CD23 gene, has been associated with differences in glycosylation, response to inhaled steroid in a variety of clinical trials including the NHLBI CAMP study, and in the potential association with total IgE serum levels.

In addition, differential signaling pathways, that CD23 may utilize have been analyzed by our group utilizing primary tonsil B cells, in vitro B cell lines, as well as peripheral blood mononuclear cells, monocytes and the monocyte cell line U937. Activation of the tyrosine kinase Fyn, and AKT is observed only in B cells not in monocytes although both cell types eventually activate IKK kinase and MAP kinase. These results suggest that the signal transduction pathway initiated by engagement of CD23 may be different in different target cells and tissues, and may affect the expression of allergic diseases in different tissues. The molecular association of the R62W SNP that alters the glycosylation and turnover of CD23 may have a significant affect on signaling pathways. Studies that delineate the contribution of the SNP variant (R62W) are being investigated in detail, and these signaling models will be reported.

Abstracts

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Oral mite anaphylaxis (the pancake syndrome)

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Oral mite anaphylaxis (OMA) is a new syndrome characterized by severe allergic manifestations occurring in atopic patients shortly after the ingestion of mite-contaminated foods, especially those prepared with wheat flour. It is observed more often in tropical countries and related with pancake meals. An increased prevalence of this clinical form of allergy is present in patients with urticaria and angioedema due to non steroidal anti-inflammatory drugs (NSAIDs).

Among mite species involved in the production of OMA various groups have observed domestic mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), as well as storage mites (*Suidasia spp*., *Aleuroglyphus ovatus*, *Lepidoglyphus destructor*, *Tyrophagus putrescientiae*, *Tyreophagus entomophagus*, *Blomia tropicalis* and *Blomia freemani*). We have demonstrated that mite termorresistant allergens are responsible for the triggering of the symptoms.

More recently, a related clinical picture of exercise-induced OMA has been observed and designated as Mite ingestion-associated Exercise-induced Anaphylaxys.

Diagnosis of OMA is based on the following criteria:

1. Presence of allergic symptoms occurring after the intake of foods prepared with wheat flour, mainly pancakes.
2. Previous history of atopic diseases (rhinitis, asthma, dermatitis, food allergy).
3. Demonstration of IgE-mediated sensitization to mites.
4. Positive immediate-type skin tests with extract from the suspected flour.
5. Negative skin tests with wheat and uncontaminated flour extracts.
6. Clinical tolerance to uncontaminated flour.
7. Microscopic identification of mites in the suspected flour.
8. Presence of mite allergens in the flour.
9. Cutaneous hypersensitivity to NSAIDs.

Prophylaxis of this syndrome consists of storing wheat flours in sealed containers in the refrigerator, since low temperatures prevent mite proliferation in the flour.

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IgG1, IgG4 and IgE antibodies to nasopharyngeal colonising bacteria in the development of atopy and asthma

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The immune responses to conserved antigens of nasopharyngeal colonising bacteria provide an avenue to study specific immunity to antigens encountered at the respiratory mucosae and how they interact with or provide prognostic markers for allergy and allergic disease. They could provide insights into the known relationship between susceptibility to bacterial infection and allergy. This report analyses IgE and IgG subclass antibodies titres made with purified recombinant antigens and absolute quantitation. Results from two cohorts show that children prone to asthma exacerbations or recurrent hospitalisation for asthma have markedly decreased IgG1 antibodies to conserved outer membrane protein antigens of the respiratory colonising bacteria Haemophilus influenza and Streptococcus pneumoniae. The low responses have their genesis in infancy as shown by their delayed development to multiple antigens of both of these bacteria in infants who later become skin test positive and develop asthma. About 30% of atopic but not non-atopic school-age children and adults also show the Th2-dependent IgG4 antibody although the presence of this isotype is not over-represented in severe disease. The IgG4 develops early in life in atopic and non-atopic infants but decreases over the next 5 years with significantly faster declines in non-atopic children. IgE antibodies to the bacterial antigens are, depending on the antigen, present in 40-50% of the population regardless of atopy. Although the IgE titres can increase to high levels after asthma exacerbation their presence and titre in atopic children are paradoxically highly associated with a decreased risk of asthma. This appears to define an interesting phenotype where the production of IgE to different bacteria and their different antigens is highly correlated but not related to atopy. In conclusion the antibody responses to conserved antigens of bacteria that colonise the respiratory mucosa show marked and surprising differences associated with atopy and asthma and these arise before the development of allergy and allergic disease.

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β2-adrenergic receptor haplotypes and asthma in childhood

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Background Genetic polymorphisms in the beta2 adreno-receptor (*ADRB2*) gene have been associated with asthma, but associations with early childhood asthma phenotypes are unclear.

Objective: To determine associations between asthma development and *ADRB2* polymorphisms during the first 10 years of life.

Methods: Three single nucleotide polymorphisms (SNPs) at codons 16, 27 and 164 in the *ADRB2* gene and one SNP upstream of the coding block (-19) were genotyped in 953 children from the birth cohort "Environment and Childhood Asthma" study. *ADBR2* SNPs and haplotypes were analyzed for association with asthma traits at birth (tidal breathing flow volume (TFV) parameters), 2 years (recurrent bronchial obstruction (rBO), TFV parameters, bronchodilator response and allergic sensitisation), and 10 years of age (flow-volume loops, bronchial hyperresponsiveness (BHR) and allergic sensitisation) and a structured interview determining asthma history.

Results At least one copy of haplotype 5`TGCC`3 was in the logistic regression analyses associated with 44 percent reduced risk of rBO at two years (OR=0.56, 95% CI 0.326, 0.957, p=0.033) and 45 percent reduced risk of current asthma at 10 years (OR= 0.554,

95% CI 0.356, 0.862, p=0.009). Additionally, at least one copy of haplotype 5'CGGC'3 reduced the risk of forced expiratory volume in the first second <80% of predicted (OR=0.285, 95%CI 0.109, 0.742, p=0.006). *ADBR2* haplotypes were not associated with the remaining phenotypes.

Conclusion A specific *ADRB2* haplotype was associated with decreased risk of asthma at two and 10 years of age, but the effect was not exerted through modulation of bronchodilator response, BHR or allergic sensitisation.

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Flame retardant BDE-153 stimulates adaptive immunity to inhaled allergens by activation of dendritic cells

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Dendritic cells (DCs) are the most potent antigen presenting cells and have to interpret the inflammatory context in which an antigen is encountered. The role of environmental pollutants in activation of DCs leading to sensitisation to allergens is not clear. Over the last decades brominated flame retardants (FR) have been added to materials, such as furnishings, electrical devices and interior decoration, to achieve the fire safety regulations. Brominated flame retardants have been detected in human serum. One main route of exposure is via inhalation, particularly via house dust.

Brominated flame retardants could act on DCs in several direct or general pathways. We examined the effect of flame retardant BDE-153, one of the most common flame retardants detected in house dust, on the maturation of DCs and the subsequent outcome of the immune response to inhaled allergens in a dendritic cell driven model for asthma. Bone marrow derived DCs were exposed to a model allergen Ovalbumin (OVA) and simultaneous to flame retardant BDE-153 (10 μM) or the solvent *in vitro*. Flame retardant exposed DCs induced upregulation of especially the costimulatory molecule CD86 and in lesser extent of CD80 and CD40. These DCs were administered intratracheally in mice to induce sensitisation to OVA. Two weeks later mice were challenged with the same allergen i.n. Preliminary results showed that mice sensitised with OVA pulsed DCs exposed to flame retardant BDE-153 developed a more severe eosinophilic airway inflammation compared with mice sensitised with control OVA pulsed DCs. More eosinophils were recruited into the bronchoalveolar space (22.2%±6.0 vs 15.3%±7.6) although not significantly. Ex-vivo restimulation of the lung draining lymph nodes with OVA induced significant higher IL-4 (p=0.032) and a trend of higher IL-5 and IL-13 (p=0.063) production. The IgE/IgG1 ratio in serum was increased twice in the BDE-153/OVA DC sensitised group compared with the control OVA/DC group although not significant (p=0.095). Airway histology slides stained with PAS showed a slight non-significant increase in severity of mucus production and eosinophilic peribronchial infiltration. Further research will have to confirm whether brominated flame retardant BDE-153 stimulates the adaptive immune response to inhaled OVA via maturation of DCs.

POSTER SESSION 2: Mast Cells, Basophils, Eosinophils, Dendritic Cells and T Cells

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The influence of isoforms of the major birch pollen antigen Bet v 1 on the activation of dendritic cells and T cell differentiation

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Previously, defined naturally occurring isoforms of allergenic proteins were classified as hypoallergens and therefore suggested as agent for immunotherapy in the future. In the present manuscript we report for the first time the molecular background of hypoallergenicity by comparing the immunological behavior of hyperallergenic Bet_v_1a and hypoallergenic Bet_v_1d, two isoforms of the major birch pollen allergen Betula verrucosa_1. Despite their cross-reactivity, Bet_v_1a and Bet_v_1d differ in their capacity to induce protective antibody responses in Balb/c mice. Both isoforms induced similar specific IgE levels, but only Bet_v_1d expressed relevant titers of serum IgGs and IgAs. Interestingly, hypoallergenic Bet_v_1d activated dendritic cells more efficiently, followed by the production of increased amounts of Th1 as well as Th2 type cytokines. Surprisingly, compared to Bet_v_1a, Bet v 1d immunized mice showed a decreased proliferation of regulatory T cells. Crystallographic studies and dynamic light scattering revealed that Bet_v_1d demonstrated a high tendency to form disulfide-linked aggregates due to a serine to cysteine exchange at residue 113. We conclude that aggregation of Bet_v_1d triggers the establishment of a protective antibody titer and supports a rationale for Bet_v_1d being a promising candidate for specific immunotherapy of birch pollen allergy.

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Inducible nitric oxide synthase expression and nitric oxide production in mouse bone marrow-derived mast cells

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Nitric oxide (NO) is important signaling molecule that regulates mast cell (MC) function. However, there are discrepancies in the literature about whether MC express isoforms of nitric oxide synthase (NOS) and make NO. MC progenitors mature in peripheral tissue under microenvironment influences. Although there has been remarkable progress in *in vitro* culture of MC from progenitors, the factors that influence MC maturation are not fully understood and our ability to control the phenotypes of MC *in vitro* and their maturation is limited.

We hypothesized that NOS expression and NO production in MC are finely controlled, and that the microenvironment conditions involved in MC maturation can play an important role in whether or not MC express NOS and produce NO. To test this hypothesis about microenvironmental conditions that are "permissive" for NOS expression, we cultured bone marrow-derived MC (BMMC) of *Balb/c* mice with various culture conditions, eg., IL-3, SCF+IL-3, or SCF+IL-4 for >3 weeks and then examined NOS expression. We detected inducible NOS (iNOS) mRNA by RT-PCR in BMMC cultured with SCF+IL-4 but not IL-3 or SCF+IL-3 and iNOS protein expression was detected by western blot and confocal microscopic analysis. After stimulation with IFN-γ (5ng/ml) in the presence or absence of IgE/Ag (antigen) in BMMC cultured with SCF+IL-4, but not in BMMC cultured with other conditions, NO production was detected using the Griess assay. Confocal microscopic analysis

showed that the iNOS expression induced by IFN-γ colocalized in $CD117^+$ ($c\text{-}kit^+$) BMMC. NO production after activation with IFN-γ in BMMC cultured with SCF+IL-4 was abrogated (>75 % inhibition) by pretreatment with the iNOS specific inhibitor, 1400W (5μM), partially blocked by the general NOS inhibitor, L-NMMA (5μM), but not blocked by the eNOS selective inhibitor, L-NAME (5μM). We further investigated whether BMMC cultured with IL-3 (>3 weeks) can be permissive for NOS expression when further cultured with SCF, IL-4, or SCF+IL-4 in the presence of IL-3. Additional culture of BMMC up to 4 weeks with these cytokine cocktails could not induce a phenotype of BMMC permissive to NOS expression after IFNγ treatment with or without IgE/Ag.

These results suggest that MC progenitors that develop in certain permissive microenvironments such as SCF+IL-4 can be induce iNOS after receiving appropriate signals such as IFN-γ and subsequently produce NO. This NOS^+/NO^+ MC phenotype is likely to be functionally distinct from MC unable to express NOS or make NO.

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Crosstalk of interleukin-4, stem cell factor, and immunoglobulin E-dependent activation in mature human mast cells

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Background: Although, crosslinking of the high affinity immunoglobulin (Ig) E receptor (FcεRI) is the major trigger for mast cell activation, cytokines like stem cell factor (SCF) and interleukin (IL) -4 are also identified as important regulators of human mast cell activation. IL-4 acts as priming factor by strongly enhancing SCF-dependent proliferation and IgE–dependent mediator release. A combined stimulation of mast cells by FcεRI crosslinking and SCF could also amplify the mediator release. Here, we analyzed the intracellular crosstalk of IL-4, SCF, and IgE-dependent activation in human intestinal mast cells.

Methods: Mature human mast cells were isolated from surgical tissue specimens by combined mechanical and enzymatic tissue dispersion and purified using MACS-technique. Mast cells were cultured in the presence of SCF with or without IL-4 for 10 days. Cells were stimulated with SCF and/or by FcεRI crosslinking using IgE/anti-IgE. Release of mediators was analyzed by ELISA, cytokine mRNA expression by real-time RT-PCR, and activation of intracellular kinases by Proteome Profiler™ array.

Results: We found a potentiated release of histamine, β-hexosaminidase, leukotriene C_4, IL-5, and IL-8, but not IL-6, in response to combined treatment with IL-4, SCF, and FcεRI crosslinking. Analyzing the phosphorylation status of intracellular kinases as mitogen activated protein kinases (MAPK) or protein kinase B (PKB; synonym Akt) we observed that activation with SCF alone induced phosphorylation of MAPK extracellular signal-regulated kinase (ERK) and p38, as well as PKB. Crosslinking of FcεRI alone resulted in activation of ERK and p38, but not PKB, whereas pre-treatment with IL-4 followed by IgE/anti-IgE triggering induced activation of PKB. Interesting, combined treatment with IL-4, SCF, and FcεRI crosslinking dramatically up-regulated activation of PKB. Furthermore, priming with IL-4 caused an increased activation of ERK, but blocked activation of p38.

Conclusion: IL-4 or SCF, respectively, are necessary for the phosphorylation of PKB in antigen-stimulated mast cells. Furthermore, SCF induced activation of p38, whereas IL-4 priming inhibited it.

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Differential T cell responses after exposure of dendritic cells to Bet v 1 isoforms or homologous food allergens

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Background: The major birch pollen (BP) allergen Bet v 1 is the most relevant sensitizing protein for BP allergic individuals. Bet v 1 is present in pollen as a mixture of at least 14 isoforms that share high sequence and structural identities. Bet v 1 homologues from plant foods are mainly involved in allergic reactions as a consequence of IgE cross-reactivity.

Objective: To examine the polarisation of the Th cell response after exposure of dendritic cells (DCs) to Bet v 1.0101, Bet v 1.0401, Bet v 1.1001, Mal d 1, Api g 1, or Dau c 1.

Methods: Immature monocyte-derived DCs (MoDCs) were generated from peripheral blood monocytes of BP allergic and healthy donors by culture with GM-CSF and IL-4 and subsequently pulsed with the allergens in combination with the maturation factors TNF-α and IL-1β.Cell surface markers were analyzed by FACS. Mature DCs were co-cultured with autologous naïve Th cells and T cell proliferation and cytokine profiles were examined.

Results: MoDCs from BP allergic and non-atopic individuals stimulated with Bet v 1.0101, Bet v 1.0401, Bet v 1.1001, Mal d 1, Api g 1, or Dau c 1 in combination with the maturation factors resulted in a mature DC phenotype in both donor groups as measured by costimulatory molecule up-regulation. Only Bet v 1.0101-stimulated MoDCs from allergic subjects enhanced the proliferation of autologous Th cells and the expression of the Th2 cytokines IL-5 and IL-13. Api g 1-primed MoDCs, in contrast, not only significantly enhanced the production of the Th1 cytokine IFN-γ but also down-regulated IL-13. Bet v 1.0401, Bet v 1.1001, Mal d 1, and Dau c 1 were not able to significantly induce Th cell proliferation or polarization. MoDCs of healthy donors failed to induce a significant proliferative response with only low levels of polarizing cytokines produced.

Conclusions: These results provide evidence that DCs discriminate between allergens, highly related isoforms and homologous proteins. This suggests the existence of distinct features of Bet v 1.0101 to act as the sensitizing agent in the birch pollen-food syndrome.

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IL-4 influences TH2 development via STAT6-dependent downregulation of negative acting T-Cell Factor 1 isoforms in peripheral T cells

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T-Cell Factor-1 (TCF-1) is a Wnt pathway transcription factor known to play essential roles in various developmental processes. Its function in T cell development in the thymus is well characterised, but little is known about its role in mature peripheral T cells. TCF-1 is still expressed at this later stage in T cell development, but is down-regulated upon T cell receptor stimulation. A recent study has demonstrated that TCF-1 initiates T_H2 differentiation by inducing the transcription factor GATA-3, indicating a crucial role for TCF-1 in mature T cells. We have investigated the role of TCF-1 in peripheral T-cells in order to analyse a potential role for the induction of allergy. Since IL-4 is the crucial cytokine for T_H2 differentiation, we have focused on understanding effects of IL-4 on TCF-1 expression. There are several functionally different isoforms of TCF-1 and one way to achieve specific effects would be to differentially regulate these isoforms. This hypothesis could indeed be confirmed.

In agreement with the association between T_H2 development and TCF-1, we have observed using qRT-PCR that TCF-1 expression is down-regulated by IL-4, whereas IL-12 does not affect TCF-1 expression. The inhibitory effects of IL-4 were predominantly observed on the shorter, dominant negative TCF-1 isoforms, while the longer, fully functional isoforms seem to be less inhibited. Many IL-4 induced effects are mediated through the transcription factor STAT6. The interaction of STAT6 with four different STAT6 binding sites which were identified within the human TCF-1 genomic locus was shown by EMSA studies. The crucial role of STAT6 in IL-4 mediated TCF-1 suppression was ascertained when we analysed T cells derived from STAT6 deficient mice. Control T cells strongly down-regulated TCF-1 in response to IL-4, whereas TCF-1 expression in STAT6 deficient T cells was not affected by IL-4. This finding was confirmed by STAT6 knockdown experiments in human peripheral T helper cells. In brief, this study demonstrates that IL-4 preferentially inhibits shorter TCF-1 isoforms in a STAT6 dependent way, thereby mediating the balance between stimulatory and inhibitory TCF-1 isoforms. The proper balance in the expression of isoforms may indeed be more relevant to the effects of TCF-1 in mature T cells than the overall expression level. This study also demonstrates that STAT6 is capable to fine-tune gene expression in more sophisticated ways than on/off-regulation.

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The allergic effector unit: Mast cell-eosinophil paracrine and physical interactions regulate cell survival

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Mast cells (MCs) and eosinophils (Eos) are the key effector cells of the allergic reaction. Though classically associated with different stages of the response, the cells co-exist in the inflamed tissue in the late and chronic phases in high numbers, and are likely to cross-talk. While some MC mediators have been shown to affect Eos biology and vice versa, the nature and functionality of possible physical interactions between MCs and Eos has not been described as of yet. We therefore aimed to investigate whether an intercellular MC-Eos "Allergic Effector Unit", comprised of bi-directional effects, could exist in an allergic setting and have a consequence on cell viability. Tissue sections from human and murine allergic disorders were specifically stained histologically and by immunohistochemistry for both MCs and Eos. In addition, co-cultures of human cord blood-derived MCs and peripheral blood Eos were carried out in various conditions and cell:cell ratios, and viability was assessed by PI exclusion in flow cytometry. We have observed close MC-Eos pairs in nasal polyps of allergic rhinitis patients and bronchi of asthmatic patients. Moreover, high rates of interacting MCs and Eos were detected in the skin of mice with ovalbumin-induced atopic dermatitis. *In vitro,* Eos displayed substantially increased survival rates following long-term (72h) co-cultures with MCs in SCF-containing media, an effect observed even in allergic inflammation mimicking MC:Eos ratios (1:5-1:10). Analysis of full contact co-cultures vs. partial (transwell) co-cultures revealed that the MC-induced Eos survival involves paracrine communication (likely mediated by increased GM-CSF release in co-culture), but also required direct cell membrane interactions, probably via surface receptor-ligand binding. Our findings suggest that a network of paracrine and physical MC-Eos interactions could serve to enhance the allergic response by augmenting Eos viability. This effect, together with other functional interplay between the cells, may thus critically modulate late/chronic inflammatory reactions and may be a good target for therapeutic intervention in allergic diseases.

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Omalizumab is also effective in non autoimmune urticaria

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Rationale: Successful treatment of chronic urticaria (CU) with Omalizumab has recently been reported. It is believed that Omalizumab acts by reducing free IgE levels with a subsequent decrease in Fc_eRI expression on mast cells and basophils thus limiting cross-linking of IgE receptors by IgG autoantibody. Patents' basophils have also been reported to be hypo-responsive to rabbit IgG anti IgE antibody.

Objective: We propose to treat patients with CU having no evidence of autoantibodies (i.e. chronic idiopathic urticaria), to determine whether Omalizumab would have any effect on symptoms and whether any effect on patients' basophil responsiveness would be evident.

Methods: Five patients were chosen with severe chronic urticaria who were unresponsive to antihistamines at high doses and also refractory to low-dose corticosteroid and cyclosporine. Antithyroid antibodies were not present and the patients' sera were negative for IgG antibody to the IgE receptor based on basophil histamine release and expression of CD63. Patients were treated with Omalizumab; we administered 300mg of Omalizumab to all patients.

Results: Two patients became asymptomatic after two doses. The Omalizumab was discontinued and the urticaria did not recur during a 7 and 18 month respectively observation period. Three patients also responded dramatically after two doses but required a maintenance dose every three months during which time they were minimally symptomatic. Four patients were hypo-responsive to rabbit anti IgE antibody and this abnormality reversed in two patients after 2 and 7 months of treatment. Basopenia was observed in all patients and reversed in 1 subject.

Conclusion: Omalizumab can affect the course of chronic urticaria, dramatically in some instances, even in the absence of anti IgE receptor antibody. It is proposed that lowing IgE and IgE receptor levels down-regulates inflammation involving cells bearing these molecules which leads to marked improvement in urticaria.

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The bacterial quorum sensing molecule, N-(3-oxododecanoyl)-L-homoserine lactone, attenuates mast cell responses to stimuli via a nitric oxide dependent mechanism

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Bacteria communicate with each other through a cell density dependent signaling system termed quorum sensing. It is becoming evident that certain components of the quorum sensing system can also influence the behavior of eukaryotic cells. In particular, emerging data suggests that acyl homoserine lactones (AHLs), a class of quorum sensing molecules produced by gram-negative bacteria, can modulate mammalian cell responses. Mast cells are at the frontline of host defense and can translate signals between the immune, nervous and endocrine systems. Thus these cells are ideally placed, anatomically and functionally, to intercept and relay bacterial signals to the host. In the current study we determined the ability of AHLs to modulate mast cell responses to stimuli.

Methods: Murine bone marrow derived mast cells (BMMC) were used to determine the effect of a range of AHLs on mast cell responses to LPS, peptidoglycan, and IgE mediated stimulation.

Results: Two AHLs tested, N-decanoyl-L-Homoserine lactone and N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL), attenuated BMMC degranulation and release of cytokines in response to FcεR1 aggregation. This occurred in a concentration dependent manner and was not a result of apoptosis, as determined by Annexin V labeling. These AHLs also inhibited LPS and peptidoglycan induced IL-6 and TNF production. 3OC12-HSL, a major component of the Pseudomonas aeruginosa quorum sensing system was the most potent inhibitor. 3OC12-HSL at 10 μm reduced maximal antigen induced β-hexosaminidase release by 65.4 ± 4.5 % and LPS induced IL-6 and TNF release by 72 ± 3.4% and 75 ± 4.2 % respectively (n=6, $p<0.001$). Evidence suggests that nitric oxide has an important role in mediating these effects as exposure of cells to 3OC12-HSL led to an increase in intracellular nitric oxide while the NOS inhibitor, L-NAME, abolished the effect of 3OC12-HSL on cell responses.

Conclusions: AHLs represent a novel mode of interaction between bacteria and mast cells. It is possible that through its action on mast cells 3OC12-HSL can inhibit host immune responses and may contribute to the pathogenicity of the Pseudomonas aeruginosa. A greater understanding of this novel form of interkingdom signaling may identify new strategies for the prevention and treatment of bacterial infection.

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Control of human basophil activation by the SH2-containing inositol 5-phosphatase (SHIP)-1 is dependent on the nature of high-affinity IgE receptor engagement

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Human basophils rapidly generate and release important pro-inflammatory and immunomodulatory mediators upon high-affinity IgE-receptor crosslinking with allergens or anti-IgE. Here, we show that the ability of basophils to respond to IgE-mediated triggers is negatively associated with the level of constitutive SHIP-1 phosphorylation as well as the type of IgE-dependent stimulus employed. Basophils were obtained from buffy coats and highly purified by negative selection and magnetic cell sorting. Cells were incubated with a variety of IgE-dependent triggers together with unstimulated controls for varying periods in order to determine rate and magnitude of histamine release and intracellular signalling (by Western blotting). We observed that constitutive SHIP-1 phosphorylation (but not total SHIP-1 or Syk expressions) in unstimulated basophil preparations correlated closely with maximum basophil responses in terms of histamine release to anti-IgE. SHIP-1 was also associated with limiting basophil responses following supraoptimal concentrations of IgG-anti-IgE, a crosslinking agent that produced striking bell-shaped dose response curves. In contrast, there was no marked reduction in histamine release or increased SHIP-1 phosphorylation following supraoptimal stimulation of basophils with either concanavalin A or IgM-anti-IgE antibodies. The kinetics of basophil activation were also more rapid for the latter stimuli. These data show that overall basophil sensitivity to IgE-dependent activation depends on reduced constitutive SHIP-1 control but the pattern of dose-response curves generated by various IgE-mediated triggers differentially involves SHIP-1 input, depending on the properties of the crosslinking agent, such as IgE-binding affinity and valency.

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Induction of airway hyperreactivity depends on a subset of iNKT cells that express IL-17RB and can be inhibited by a CD1d-dependent antagonist

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The prevalence of asthma continues to increase in westernized countries, and optimal treatment remains a significant therapeutic challenge. IL-25 has been shown to induce Th2 responses and airway-hyperreactivity, a cardinal feature of asthma, but the mechanism of action is not understood and it is unclear which cells mediate this disease. Recently, CD1d-restricted *i*NKT cells were found to play a critical role in the induction of airway hyperreactivity in animal models and are associated with asthma in humans. Here we show that the receptor for IL-25, IL-17RB, is highly expressed on a subset of naïve and activated $CD4^+$ iNKT cells, but not on activated T cells. IL-$17RB^+$ iNKT cells produced large amounts of Th2 cytokines, which was substantially increased by IL-25 stimulation. Furthermore, IL-$17RB^+$ iNKT cells were capable of restoring AHR in iNKT cell deficient mice, whereas IL-$17RB^-$ iNKT cells failed to reconstitute AHR and lung inflammation.

To test whether *i*NKT cell-targeted therapy could be used to treat allergen-induced airway disease, mice were sensitized with ovalbumin and were treated with DPPE-PEG, a CD1d-binding lipid antagonist. A single dose of DPPE-PEG prevented the development of airway hyperreactivity (AHR) and pulmonary infiltration of lymphocytes upon ovalbumin challenge, but had no effect on the development of ovalbumin-specific Th2 responses. In addition, DPPE-PEG was able to completely prevent the development of AHR after

administration of α-GalCer intranasally. Since *i*NKT cells play a critical role in the development of AHR, the inhibition of *i*NKT activation by DPPE-PEG suggests a novel approach to treat *i*NKT cell-mediated diseases such as asthma.

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Endogenous IL-10 helps limit excessive Th2 responses in peanut allergic humans yet plays little role in maintaining clinical tolerance in non-allergic individuals

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Background: Despite ubiquitous exposure, 97-99% of the population maintains lifelong clinical tolerance to peanut and other dietary antigens. The mechanisms controlling the immune-regulatory cytokine responses characteristic of clinically tolerant vs food allergic individuals remain obscure. Several murine and human systems argue for a role of elevated IL-10 production in impeding or redirecting Ag-specific cytokine responses to achieve clinical tolerance.

Objective: Assess the roles played by endogenous IL-10 in regulating human Ag-specific cytokine recall responses to peanut. Determine if peanut allergic and clinically tolerant populations differ in their production of, or response to, this cytokine.

Methods: >100 clinically well characterized individuals ranging in age from 2-45y were studied. Fresh mononuclear cells were stimulated with peanut Ag directly ex vivo in primary culture to assess Type 1, Type 2, and regulatory cytokine response profiles, relative levels of IL-10 production and the impact of blocking endogenous IL-10 (and/or TGFb) on Ag-dependent recall responses.

Results: IL-10 production elicited by peanut Ag specific stimulation was higher in peanut allergic rather than non-allergic individuals. While addition of exogenous rIL-10 was equally effective at abrogating peanut-driven cytokine responses in both populations, blocking of endogenous IL-10 function in vitro had the most striking effects on responses in peanut allergic individuals. Blocking both IL-10 and TGFb function clearly enhanced both type 1 and type 2 recall responses amongst allergics, but had limited, sporadic effect on cytokine production by the non-allergic population.

Conclusion: The data argue against a role for IL-10 and TGFb as an essential mechanism of oral tolerance to dietary antigens. Peanut non-allergic individuals exhibit both lower levels of Ag-driven IL-10 production, and lesser impact on food Ag driven recall cytokine production upon its neutralization. Conversely, elevated peanut stimulated IL-10 production found among individuals with established histories of peanut allergy may indicate a relevant control mechanism attempting to inhibit the intensity of undesirable "allergic/Th2" and pro-inflammatory (IFNg) cytokine expression in such individuals.

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A Th1/Th2-associated chemokine imbalance preceding allergic disease is influenced by birthsize, breastfeeding, daycare and probiotics

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Background: Analyses of circulating chemokines offer novel tools to investigate the Th1/Th2 imbalance in allergic disease *in vivo* and explore the influence of pre- and postnatal factors in infancy.

Objective: To relate circulating Th1- and Th2-associated chemokines to the development of allergic disease, pre- and postnatal factors and probiotic supplementation in infancy.

Methods: Circulating levels of Th1-associated CXC-chemokine ligand (CXCL)9, CXCL10 and CXCL11 and Th2-associated CC-chemokine ligand (CCL)17, CCL18 and CCL22 were assessed with Luminex and ELISA at birth (n=109), 6 (n=104), 12 (n=116) and 24 months (n=123) in infants completing a double-blind placebo-controlled allergy prevention trial with *Lactobacillus reuteri* during the last month of gestation and through the first year of life. The infants were followed regarding development of allergic disease and sensitization until two years of age.

Results: The Th2-associated chemokines were as highest at birth and then decreased, whereas the Th1-associated chemokines increased with age. Low Th1- and high Th2-associated chemokine levels were observed in children developing allergic disease. Sensitization was preceded by elevated CCL22 and reduced CXCL11 levels. High Th2-associated chemokine levels correlated positively to birth length and weight and breastfeeding, and high Th1-associated chemokine levels to daycare. Prevalence of *L. reuteri* in stool the first week of life was associated with low CCL17 and CCL22 and high CXCL11 levels at 6 months.

Conclusion: Allergic disease in infancy was associated with low circulating Th1- and high Th2-associated chemokine levels during the first year of life. The chemokines levels were affected by both pre and –postnatal factors.

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Human CD8 T-cells initiate and sustain Th1 responses by promoting IL-12p70 and differentiation of blood monocytes into TNF-α/iNOS-producing dendritic cells

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CD8 T cells are known to contribute to the induction of T-helper 1 (Th1) inflammatory responses. However, the underlying mechanisms are not fully understood. Here, we show that human CD8 T cells interact with dendritic cells (DCs) to initiate and sustain Th1 responses by secreting a combination of cytokines and induce the rapid development of inflammatory TNF-α/inducible Nitric Oxide Synthase (iNOS) (Tip)-DCs. We show that activated CD8 T cells initiate this response by secreting IFN-γ to prime dendritic cells (DCs) for efficient IL-12p70 production. CD8 T cells sustain the Th1 response during their interaction with DCs by creating a cytokine milieu, containing high levels of GM-CSF, IL-1, IL-2, IL-6, IFN- γ, and TNF-α, that promote the differentiation of peripheral blood monocytes into Tip- DCs. These Tip-DCs expressed the C-type lectin receptor CD209 and increased levels of CD40, CD80, CD86 and the maturation marker CD83. Tip-DCs also exhibit a reduced

capacity to phagocytose bacteria but were able to prime naive CD4 T cells for proliferation and IFN-γ production. This study identifies a mechanism by which human CD8 T cells can rapidly and effectively induce and sustain Th1 immunity.

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H3/H4 histamine receptors and IgE regulation in healthy donors and allergic patients

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Background: Previously it have been shown that several histamine H_3/H_4 receptor antagonists modulate IgE synthesis in atopic and non-atopic diseases (R. Khanferyan et al., 2003-2009). This investigation evaluates the IgE-modulatory activity of the highly specific agonist of H_3/H_4 histamine receptors - R-α-methyl [^{3}H] histamine (RMH) as well as the H_3/H_4 antagonist with high affinity (Ki=0.26 nM)- Imoproxifan (IMP) on IgE response in healthy donors and ragweed sensitive patients.

Methods: Supernatants obtained after 9-days culture of peripheral mononuclear blood cells (PBMC), from either healthy donors (10) or from allergic subjects sensitive to ragweed pollen (12) during and out of the pollen season, were assayed for total IgE by CAP FEIA method (Phadia). $H_{3/4}$ receptor agonist RMH as well as IMP were added to the cultures. For the specific allergen stimulation of PBMC, ragweed allergen (Greer, USA) at two different concentrations of AgE was added to the cultures of PBMC.

Results: RMH at high (10^{-5}M) concentration suppressed spontaneous IgE synthesis by PBMC from healthy donors up to 30%. Both concentrations (10^{-5}M and 10^{-8}M) of RMH added to PBMC pre-stimulated by low concentrations of ragweed allergen (1 IU of Antigen E) induced lower IgE synthesis while PBMC pre-exposed to higher concentrations of allergen (10 IU of AgE) after cultivation with RMH produced elevated levels of IgE. IMP in healthy donors increased an IgE synthesis and diminished IgE-supressory activity of the high concentration of histamine. Out of pollen season IMP showed most pronounced effect on histamine induced IgE synthesis. At the same time, IMP together with histamine (10^{-5}M) developed co-suppressory effect on the IgE synthesis in PBMC of patients cultured during pollen season. IgE-modulatory effects of $H_{3/4}$ receptor antagonist highly-dependent on IL4, γ-IFN and IL10, rather than IL13 synthesis.

Conclusion: Histamine $H_{3/4}$ receptors are involved in the modulation of IgE synthesis in healthy donors as well as in allergic patients. In allergic patients, enhanced production of IgE by PBMC related to activation or suppression of $H_{3/4}$ histamine receptors depends on the magnitude of PBMC cell activation which is directly related to the level of allergen exposure.

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Both CD4+ and CD8+ T cells in skin of atopic dermatitis and psoriasis patient are potent cytokine releasing cells, each disease with its specific T cell cytokine profile

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Introduction: Atopic dermatitis (AD) and psoriasis are T cell-mediated chronic inflammatory skin disorders. In general, AD skin T cells are supposed to be of the Th2/Th1 phenotype, whereas in psoriasis the local T cells are predominantly regarded as Th1/Th17 cells. Research on T cells in AD has largely ignored the potential role of $CD8^+$T cells.

Aim: To investigate and compare the phenotypes of T cells present in lesional and non-lesional skin of AD and psoriasis patients using a novel model of short-term cultures of skin-derived T cells (R.A. Clark, JID 2006).

Methods: Biopsies from lesional and non-lesional skin were minced and placed on collagen pre-incubated grids. After 2 and 3 weeks T cells were isolated and phenotypes were determined by flowcytometric analysis of membrane markers. For selected samples this was compared to microscopic evaluation of immunohistochemical stained skin samples obtained at t=0. The cytokine producing capacity was determined by flowcytometric analysis of PMA/Ionomycin-stimulated T cells.

Results: Next to $CD4^+$T cells also $CD8^+$T cells were potent cytokine releasing cells in both psoriasis, as well as AD-skin derived T cells. Although our culture system contains low amounts of IL-15, there was no selective outgrowth of $CD8^+$T cells. In lesional AD skin increased $CD4^+$IL-4^+, $CD4^+$IL-13^+ en $CD8^+$IL-13^+ T cells were found, whereas in lesional psoriasis skin more $CD4^+$IFNγ^+, $CD4^+$IL-17^+ and $CD8^+$IL-17^+ T cells were demonstrated. The non-lesional skin of psoriasis was characterized by increased expression of IL-4 and IL-13 in both $CD4^+$ and $CD8^+$T cells, whereas in the psoriasis lesional skin more IL-17 and IFN-γ producing T cells were found. Interestingly although the number of T cells was increased in lesional AD skin compared to non-lesional AD skin, we could not find significant differences between the cytokine production profiles.

Conclusion: Next to $CD4^+$T cells in AD and psoriasis skin $CD8^+$T cells comprise an important population of cytokine releasing cells. In non-lesional skin of psoriasis a Th2/Tc2 phenotype is present, whereas in lesional skin a Th17/Tc17 and Th1/Tc1 phenotype is demonstrated.

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CCL23 production by human eosinophils

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Background: CCL23 (MPIF1/CK-BETA-8) is a novel CC-chemokine that plays important roles in inhibition of myeloid progenitor cell development, selective recruitment of resting T lymphocytes and monocytes, and potentiates VEGF-induced proliferation and migration of human endothelial cells. Since eosinophils participate in the pathogenesis of airway remodeling, we examined CCL23 production by human eosinophils in vitro.

Methods: Using Ficoll and antibody-coated immunomagnetic beads, eosinophils and other blood cells were purified from peripheral blood samples obtained from normal or mild allergic patients. Eosinophils were cultured in the presence of 10 ng/ml of GM-CSF, 10 ng/ml of IL-5, 100 ng/ml of IFN-γ or immobilized secretory IgA (sIgA). Total mRNA was extracted after 6 hours of culture, and mRNA expression was measured using a microarray and RT-PCR. The CCL23 concentration in the supernatant and cell lysate after 48 hours of culture was measured using ELISA.

Results: CCL23 mRNA was constitutively expressed in fresh eosinophils, and the expression level in the eosinophils was higher than that in other types of blood cells. CCL23 mRNA increased significantly upon stimulation with GM-CSF and IL-5, and slightly with immobilized secretory IgA. Fresh eosinophils did not contain CCL23 protein. CCL23 was significantly released into the supernatant when the eosinophils were stimulated with GM-CSF or IL-5 but not with IFN-γ or immobilized secretory IgA.

Conclusion: Our data suggested that eosinophils produce and release CCL23, and participate in some physiological and pathological conditions in vivo.

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High-resolution transcriptional profiling of chemical-stimulated dendritic cells identifies immunogenic contact allergens, but not prohaptens

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Allergic contact dermatitis is a complex syndrome and knowledge on the *in vitro* detection of small molecular weight compounds, particularly prohaptens, is limited. Therefore we investigated chemical-induced gene expression changes in human antigen presenting cells upon stimulation with immunogenic contact allergens, prohaptens and irritants.

Monocyte-derived dendritic cells (moDC) and THP-1 cells were stimulated with the prohapten cinnamic alcohol (Calc), the hapten cinnamic aldehyde (CAld), an irritant and an obligatory sensitizer *in vitro*. Whole-genome screening and consecutive PCR analysis of differential gene expression in moDC stimulated with either CAld or the obligatory sensitizer revealed co-regulation of 11 marker genes which were related to immunological reactions (IL-8, CD1e, CD200R1, PLA2G5, TNFRSF11A), oxidative or metabolic stress responses (AKR1C3, SLC7A11, GCLM) or other processes (DPYLS3, TFPI, TRIM16). In contrast, the prohapten CAlc and the irritant did not change marker gene expression. In THP1-cells, CAld and the positive control elicited similar expression changes in only 4 of the previously identified genes (IL-8, TRIM16, CD200R1, GCLM). In conclusion we provide important insights into the pathophysiological basis of ACD, identify marker genes suitable for skin hazard assessment and demonstrate that contact-allergenic prohaptens escape *in vitro* detection if their skin metabolism is not taken into account.

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Intracellular introduction of phosphorylated FcεRIβ immunoreceptor tyrosine-based activation motif inhibits IgE-dependent human mast cell activation

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Human FcεRIβ was reported to function as an amplifier of FcεRI expression and the FcRγ-mediated activation signal in FcεRI, Syk and Lyn-cotransfectant NIH 3T3 cells. We recently evaluated the expression of FcεRIβ in giant papillae obtained from patients with chronic allergic conjunctivitis, and compared the expression level of FcεRIβ with that in conjunctiva from patients with non-allergic diseases employing a specific anti-human FcεRIβ antibody generated by us. It was thereby found that the densities of mast cells were significantly increased in cases of giant papillae compared with controls ($p < 0.0001$). The ratio of FcεRIβ$^+$ cell number to tryptase$^+$ cell number in giant papillae samples (0.81 ± 0.13, mean ± SD, n = 10 donors) was significantly higher than that of the control samples (0.06 ± 0.11, $p < 0.0001$, n= 10 donors). FcεRIβ$^+$ cells were preferentially localized within and around the epithelial tissue. To clarify the role of FcεRIβ in human mast cells, FcεRIβ expression was reduced by lentiviral shRNA silencing technique. The diminution of FcεRIβ significantly down-regulated cell surface FcεRIα expression, IgE-dependent degranulation, and PGD_2 and cytokine production. The diminution of FcεRIβ inhibited the redistribution of Lyn to the cell membrane following aggregation of FcεRI. We thus developed recombinant cell-penetrating forms of phosphorylated FcεRIβ immunoreceptor tyrosine-based activation motif (pYpYpY-β) for intracellular delivery to disturb the redistribution of Lyn to the cell membrane after FcεRI aggregation. We found that the pYpYpY-β was localized in cytoplasm and captured cytosolic Lyn in human mast cells. The pYpYpY-β significantly inhibited IgE-dependent histamine release and PGD_2 synthesis, but not those induced by calcium ionophore. However, mouse pYpYpY-β had no effects on IgE-dependent degaranulation in mouse bone marrow-derived mast cells. Human pYpYpY-β did not cause any effects on IL-8 production by U937 cells after FcγRI aggregation. Thus, the pYpYpY-β may have a good potential for treatment of human allergic diseases.

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Multicistronic vector based introduction of T-cell receptor alpha and beta chains and Foxp3 or TGF-beta generates human transgenic allergen-specific T-regulatory cells

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T-cell function can be modified by ectopic overexpression of transgenes. We have shown that transfer of T-cell receptor (TCR) alpha and beta chains specific for allergen-derived epitopes can transfer allergen-specificity to peripheral blood T-cells from non-allergic individuals. Overexpression of Foxp3 has been demonstrated to result in T-cells with regulatory function. By combining these two approaches we aim to generate transgenic allergen-specific regulatory T-cells (Treg) that might become useful in immunotherapeutic approaches. cDNAs containing the respective alpha and beta chains of a TCR specific for the major birch pollen allergen epitope $Betv1_{142-153}$ (TRAV6/TRBV20) and human Foxp3 or TGFbeta linked by picornaviral 2A-sequences were cloned into multicistronic retroviral expression vectors. Transduced peripheral blood T-cells were tested for their regulatory capacity. Both Foxp3$^+$ and TGFbeta$^+$ T-cells were hyporesponsive in proliferation and cytokine secretion assays in response to polyclonal and allergen-specific stimuli,

which could be overcome by addition of exogenous IL-2. Furthermore, TCR^+Foxp3^+ transgenic T-cells inhibited T-cell proliferation of antigen-specific responder T-cells in response to allergen-specific activation. Significantly, $Foxp3^+$ transgenic T-cells expressing a non-allergen-specific T-cell receptor were unable to start their regulatory program in response to allergen-stimulation, which was in clear contrast to polyclonally activated T-cells. Thus, our results confirm that Foxp3-mediated regulation provided by allergen-specific T-cells is an activation dependent process. We show a transgenic approach to generate Treg that exert their function exclusively in response to allergen-specific activation. Such engineered allergen-specific Treg might become useful tools for tolerance-induction therapies in allergic and other immune-mediated diseases.

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Beneficial role for the tryptase mouse mast cell protease 6 in the innate immune response in the lung to pneumonia virus of mice

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Introduction: The murine mast cell protease-6 (mMCP-6), the ortholog of human tryptase-beta, is a tetramer-forming serine protease restricted to mast cells (MCs), including those that reside in the lung. While mMCP-6 has important beneficial roles in immune responses to bacterial and helminth infections, its biologic significance in viral infections of the lung is unknown. Human Respiratory syncytial virus (hRSV) infections lead to thousands of hospitalizations each year and are a major risk factor in the development of asthma. We used pneumonia virus of mice (PVM), a closely related virus that replicates the more severe sequelae of hRSV infection in inbred mice, to investigate the role of mast cell-restricted tryptase in viral infection.

Methods: Wild-type (WT) and transgenic mMCP-6-null C57BL/6 mice were inoculated intranasally with varying doses of PVM. Mice infected with low doses were monitored for clinical symptoms, including weight loss. Survival was calculated over a range of doses. Mice infected with a lethal dose were culled at the peak of clinical symptoms and airway cellularity was assessed. Viral load was measured by RT-qPCR at various time points.

Results: WT C57BL/6 mice inoculated with 25 plaque forming units (pfu) exhibited weight loss and obvious clinical symptoms including ruffled fur and hunched posture. Nevertheless, 80% of these animals survived the infection, eventually returning to their original weight. In contrast, mMCP-6-null mouse did not survive 25 pfu, and only 17% survived a dose of 6 pfu. mMCP-6-null mice also exhibited greater weight loss than WT mice at all doses. Following inoculation with 100 pfu (a lethal dose in both strains), mice were culled at the peak of clinical symptoms (6-7 days for mMCP-6-null mice or 7-8 days for WTmice). WT mice had a greater total number of leukocytes in their airways, due to increased numbers of macrophages, neutrophils and eosinophils, whereas mMCP-6-null mice had a greater viral load 1-3 days post inoculation.

Conclusion: The mast cell-restricted tryptase mMPC-6 exhibits a significant beneficial role in the innate immune response to respiratory infections with PVM. These data raise the possibility that human tryptase-beta has a similar beneficial role in hRSV infections of the lung.

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Is "MCreg" a new cell in the heaven of regulatory cells?

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CD4+CD25+ regulatory T (Treg) cells are known as modulators in mast cells (MCs) response and homeostasis. We are investigating about the role of OX40:OX40L axis, a receptor:ligand pair known to modulate Treg cell immunosuppressive activity. Interaction between OX40L on MCs and OX40 on Tregs elicited an inhibitory effect on MC degranulation, but not on IL-6 and TNF-α production through the increase of cAMP levels. Since these biological events follow the aggregation of the FcεRI receptor, we investigated whether modification in phosphorylation of proteins and mobilization of extracellular calcium are affected in presence of Treg cells to individuate molecular pathways activated by OX40:OX40L interaction that could influence MC activation. We found that the Fyn, Gab2 and Akt phosphorylation in IgE/DNP activated MCs was decreased in presence of Tregs cells but not when MCs were co-cultivated with Treg $OX40^{-/-}$ cells. Moreover, we found that MCs activated in the presence of Treg cells showed reduced Ca^{2+} influx, independently of phospholipase C (PLC)-gamma2 or Ca^{2+} release from intracellular stores, but is dependent on arrest of STIM1 traslocation, a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane. By immunofluorescence, EM localization, and surface biotinylation we demonstrate that STIM1 migration from ER-like sites to the plasma membrane upon depletion of the Ca^{2+} store is blocked in IgE/DNP activated MCs co-cultivated with Treg cells.

Moreover, we have analysed the influence of MCs/Treg interaction in presence of Teff cells and we have found that MCs provides the cytokine milieu that skew both Tregs and Teff cells into IL-17 producing T cells (Th17). All this data suggest novel molecular pathways that can be used as target of therapeutic approaches to the therapy of autoimmune and allergic diseases.

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Glycation of the model food allergen ovalbumin enhances antigen uptake and presentation by human dendritic cells augmenting allergen-specific Th2 cell responses

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Advanced glycation endproducts (AGE) of food proteins resulting from the maillard-reaction after cooking or heating may have particular importance in food allergy. The underlying immunological mechanisms are only poorly understood. The aim of the study was to analyze the effects of AGE derived from the model food allergen ovalbumin (AGE-OVA) on human dendritic cells (DC), their immunostimulatory capacity and the

T cell response compared to regular OVA. For this purpose human immature monocyte-derived DC were exposed to FITC-labeled AGE-OVA and FITC-labeled regular OVA and the uptake was analyzed by flow cytometry and fluorescence microscopy. Furthermore, autologous $CD4^+$ T cell proliferation and cytokine production induced by mature DC loaded with AGE-OVA versus OVA were compared. Finally, expression of RAGE, the receptor for AGE, and activation of the transcription factor NF-κB by AGE were investigated. Internalization of FITC-AGE-OVA by immature DC was significantly increased compared to FITC-OVA. Blocking the mannose receptor, macropinocytosis or the scavenger receptor strongly reduced uptake of both, FITC-OVA and FITC-AGE-OVA. Comparing $CD4^+$ T cells co-cultured with AGE-OVA versus OVA loaded mature DC, AGE-OVA DC produced more IL-6 and induced a stronger Th2 and weaker Th1 cytokine response while there was no difference concerning proliferation of $CD4^+$ T cells. The expression of RAGE was higher on immature DC compared to mature DC. AGE-OVA exposed immature DC showed a stronger expression of RAGE and activation of the transcription factor NF-κB compared to OVA loaded immature DC. Our data indicate that AGE-OVA may be more immunogenic/allergenic than regular OVA.

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Active transport of contact allergens in human dendritic cells and human epidermal keratinocytes is mediated by multidrug resistance related proteins

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The multidrug resistance related proteins (MRP) function as efflux transporters of a variety of large organic anions or their conjugates in hepatic detoxification, drug distribution, renal clearance and drug resistance of tumor cells. Further we demonstrated that human epidermal keratinocytes (NHEKs) and human monocytes derived dendritic cells (moDCs) express a specific pattern of efflux transport proteins especially MRPs.

Since their functional role was largely unclear, MRP-mediated efflux activity of NHEKs and moDCs was analyzed using an *in vitro* transport assay. Therefore efflux transport of radiolabeled contact allergens eugenol and isoeugenol was inhibited using the specific MRP inhibitors. Accordingly the accumulation of these substrates was determined.

Indomethacin blocked the efflux transport of [3H]eugenol up to 1.6-fold compared with control cells without indomethacin treatment. Treatment of cells with indomethacin increased intracellular concentration of [3H]isoeugenol only slightly up to 1.1-fold. Treatment of NHEKs with MK571 decreased efflux transport of [3H]eugenol and [3H]isoeugenol, respectively, to 1.6-fold. Indomethacin strongly reduced the efflux transport of [3H]eugenol up to 2.3-fold and also the efflux of [3H] isoeugenol up to 2.1-fold in moDCs.

Human DCs have been employed to assess the sensitizing potential of contact allergens and alters their cytokine gene expression profile. In particular marked IL-8 upregulation has been shown to occur during DC exposure to contact allergens. To survey the functionality of the specific MRP inhibitor indomethacin after stimulation with contact allergens IL-8 regulation was measured by a DC-based *in vitro* assay. Taqman RT PCR analysis of mRNA expression in moDCs cells after treatment with isoeugenol were conducted. Treatment of moDCs with indomethacin after stimulation with isoeugenol resulted in a 12-fold upregulation of IL-8 expression in comparison to control cells without indomethacin treatment. This contact allergen also induced DC activation indicated by upregulation of IL-8.

In addition to previous studies revealing the expression of MRPs in normal human epidermal keratinocytes and antigen presenting cells we are now providing data strongly supporting the functional role of these transport proteins in the active efflux of contact allergens in NHEKs and also in moDCs.

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Nickel (Ni) allergic patients with complications to Ni-containing joint replacement show preferential IL-17 type reactivity to Ni

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Some Ni allergic patients develop complications upon Ni-containing arthroplasty. In the periimplant tissue of such patients we had observed lymphocyte dominated inflammation together with IFNγ and IL-17 expression. We thus wondered if Ni stimulation of blood mononuclear cells (PBMC) of such patients would lead to different cytokine pattern as compared to Ni allergics with symptom free arthroplasty.

Based on history and patch testing in 15 Ni allergics (5 without implant, 5 with symptom free arthroplasty, 5 with complicated arthroplasty) and 5 non-allergics lymphocyte transformation test was performed using PBMC. In parallel in vitro cytokine response to Ni was assessed by realtime RT-PCR.

All 15 Ni allergics showed enhanced LTT reactivity to Ni ($NiSO_4$ 10^{-4}M: mean SI = 8.42±1.8, $NiSO_4$ 10^{-5}M: 4.31±1.13) compared to non-allergic control group (data not shown).The 5 healthy controls showed only marginal proliferative and cytokine response (IL-2, IL-4, IL-17, IFNγ) to Ni. Predominant IFNγ expression to Ni was found both in the 5 allergics without arthroplasty ($NiSO_4$ 10^{-4}M: 246.48±123.54, $NiSO_4$ 10^{-5}M: 36.79±12.42 fold increase vs medium control) and also in the 5 allergic, symptom-free arthroplasty patients ($NiSO_4$ 10^{-4}M: 11.36±2.33, $NiSO_4$ 10^{-5}M: 3.36±2.21 fold increase vs medium control) . In contrast, in the 5 Ni allergics with arthroplasty-linked complications a predominant, significant IL-17 expression ($NiSO_4$ 10^{-4}M: 62.40±15.35, $NiSO_4$ 10^{-5}M: 72.08±27.3 fold increase vs medium control) to Ni was seen but not in patients with symptom free arthroplasty.

The predominant IL-17 type response to Ni may characterize a subgroup of Ni allergic patients prone to develop lymphocytic periimplant hyperreactivity.

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IL-17RB$_+$NKT cell-mediated airway hypersensitivity and *IL17RB* polymorphisms associated with severity of childhood asthma

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Abstracts

IL-25 (known as IL-17E) has been reported to induce Th2-type immune responses, including increased serum IgE levels, eosinophilia, and pathologic changes in the lung and other tissues, indicating a pivotal role of IL-25 as a mediator of Th2 responses. IL-17RB^{+} NKT cells, a novel subset of NKT cells, are required for RSV- or IL-25-dependent airway hyperreactivity (AHR). NKT cell-deficient Jα18$^{-/-}$ mice failed to develop significant AHR, cell infiltration and histological changes in the lung, even after treatment with IL-25. The cell transfer of IL-17RB^{+} NKT cells, but not IL-17RB^{-} NKT cells, into Jα18$^{-/-}$ mice restored AHR and the severity of AHR depends on the cell numbers of IL-17RB^{+} NKT cells transferred.

Human *IL17RB* is located in chromosome 3p21.1. The region of around 3p21 is known to be associated with the risk of asthma by whole genome linkage analysis. Linkage disequilibrium studies were carried out with selected 8 tag SNPs. A strong association between one of the 8 SNPs, rs3017, genotype and corticosteroid dose was detected in the severity of childhood asthma. CC genotype in rs3017 was preferentially retained in children with mild asthma and SNP C was a protective allele. The protective allele, C SNP in the rs3017 genotype in the 3'-UTR of *IL17RB* create the putative binding element for a peroxisome proliferators–activated receptor (PPAR)α/γ. PPARs forms heterodimers with a retinoic acid receptor, whose complex inhibits transcription of the *IL17RB* mRNA induced by inflammatory stimuli, such as LPS, IFN-γ etc. Thus, it is likely that the mechanisms explain the association between child asthma severity and the susceptive allele of *IL17RB*.

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Influence of a tumour on mast cell number and function in human lung parenchyma

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The majority of the human lung tissue available for studying allergic and inflammatory disease is obtained from patients undergoing resection for carcinoma. These patients usually have a smoking history and may also have mild/moderate airways obstruction. It is unclear how these affect mast cell number and function. We compared the number of mast cells in macroscopically normal human lung parenchyma of 37 patients undergoing thoracic surgery in Southampton. Twenty seven patients underwent a lobectomy because of carcinoma, 11 patients with no airways obstruction (6M/5F, average age 60.7±4.4 years, FEV1/FVC=0.79±0.02) and 16 patients with mild/moderate airways obstruction (8M/8F, average age 71.4±2.1 years, FEV1/FVC=0.61±0.02). Tissue was also obtained from 10 patients undergoing bullus repair (9M/1F, average age 36.7±5.42 years). This group were significantly younger than the other two groups($P<0.001$) Tissue was embedded in GMA resin and mast cells enumerated following immunostaining for tryptase. Mast cell numbers were 48.0±24.3 cells mm^{-2} for non-cancer patients and 28.1±9.6 cells mm^{-2} in patients with no airways obstruction and 40.6±14.3 cells mm^{-2} in the patients with mild/moderate airways obstruction. We also examined the release of TNFa and IL-10 from lung tissue explants 24 hrs after stimulation with buffer control or 100μg/ml anti-IgE. There was no cytokine release in any of the groups from tissue explants stimulated with buffer alone (all groups releasing less than 1.0 pg/mg tissue). IgE dependent TNFa release was 34.8±15.4 pg/mg tissue in non-cancer patients, 51.3±14.2 pg/mg tissue in tissue from patients with no airways obstruction and 45.3±24.8 pg/mg tissue in tissue from patients with mild/moderate airways obstruction. There was no significant difference between the groups. A similar pattern was noted with IL-10 release with 3.8±1.5 pg/mg tissue released from parenchyma of non-cancer patients, 10.0±3.1 pg/mg tissue from the patients with no airways obstruction and 7.4±3.4 pg/mg tissue from the patients with mild/moderate airways obstruction.

In summary we noted a substantial variation in mast cell number and IgE-dependent cytokine release in all three groups but no significant difference between the groups suggesting that mast cell number and function is not influenced by the presence of a tumour, or the development of airways obstruction.

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Release of dipeptidyl peptidase I from human mast cells in anaphylaxis

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BACKGROUND: Dipeptidyl peptidase I (DPPI) is a cysteinyl protease that acts on the pro-forms of tryptase and chymase to generate the catalytically active enzymes that are released on anaphylactic degranulation. The extent to which DPPI may itself be released from human mast cells has been unclear.

METHODS: Mouse monoclonal antibodies (termed DD1, DD2, DD3, DD4 and DD5) and rabbit antisera were prepared against recombinant human DPPI, and their specificity confirmed by ELISA, Western blotting and immunocytochemistry. These were employed in development of sensitive enzyme-linked immunosorbent assays (ELISA) for detection of DPPI in cell lysates and supernatants, and in serum. Cells of the LAD2 mast cell line and primary cultures of mast cells from human tonsil or skin were experimentally activated with calcium ionophore A23817 or antibody specific for IgE. DPPI was measured by ELISA or using chromogenic substrates in cell lysates and supernatants. Concentrations were determined of histamine using an automated glass fibre based procedure, and tryptase and carboxypeptidase by specific ELISA. Serum was collected from patients suffering anaphylaxis, as well as from healthy atopic and non-atopic subjects. Levels of DPPI, tryptase, carboxypeptidase and histamine in the circulation were determined.

RESULTS: Immunoreactive DPPI and DPPI enzyme activity was released in a dose-dependent manner following calcium ionophore or anti-IgE induced activation of LAD2 cells or primary cultures of human mast cells. In response to both stimuli, DPPI, like histamine and tryptase, was released from cells within 5 minutes, and levels had reached a plateau by 30 minutes. Serum DPPI concentrations were significantly higher in patients with anaphylaxis than in healthy subjects who were non-atopic ($p=0.007$) or atopic ($p=0.003$). There were no differences in baseline levels in atopic and non-atopic individuals. In cases of anaphylaxis, DPPI concentrations in the circulation were correlated with those of histamine ($p=0.006$) and carboxypeptidase ($p<0.001$), but not with those of tryptase.

CONCLUSIONS: DPPI is released from human mast cells on anaphylactic degranulation, and enters the circulation in high levels in patients with anaphylaxis. DPPI may have important mediator actions in the extracellular environment, and deserves attention as a novel clinical marker of anaphylaxis.

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The role of the eosinophil in airway remodelling in asthma

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Background: While the precise role of eosinophils in asthma is still controversial it is widely accepted as a marker of responsiveness to corticosteroids and risk of exacerbation. The aim of this study was to investigate its role in airway remodelling.

Methods: Bronchial biopsies from steroid naïve asthmatics with either low (0 - 0.45 mm^{-2})(eos-low) or with high submucosal eosinophil counts (23.43 - 46.28 mm^{-2})(eos-high), and normal controls (n=20 in each group) were analysed by immunohistochemistry and computerised image analysis for the extent of epithelial damage, mucus expression and smooth muscle hypertrophy.

The extent of *in vivo* epithelial damage was quantified by immunostaining for EGFR which is up-regulated following *in vivo* damage, thereby allowing distinction from epithelium damaged artifactually during biopsy. The lengths of intact epithelium, epithelium damaged *in vivo* and artifactually damaged epithelium were measured and expressed as proportions of total epithelium.

Mucus expression was studied by staining for MUC2, MUC4, MUC5AC and MUC5B and assessing the percentage positive epithelial immunoreactivity. Sections were also stained by the PAS technique to determine goblet cell numbers.

The volume fraction of smooth muscle was assessed using stereology applied to sections stained for alpha smooth muscle actin.

Results: *In vivo* damaged epithelium (EGFR+) was significantly (p=0.02) higher in the eos-high group (27.37%) compared to the eos-low group (4.15%) and correlated positively with submucosal eosinophil numbers (r_s=0.397, p=0.002). There was no difference in the mucin expression or the number of goblet cells between the high-eos and low-eos groups. However, MUC 2 expression was increased (p=0.002) in the high-eos group (0.70%) compared to the controls (0%). Both the high-eos (33%) and low-eos (32.5%) groups had a higher proportion (p=0.046 & p=0.021) of smooth muscle compared to the controls (25.5%) but were not different when compared to one another.

Conclusion: Eosinophil counts are related to epithelial damage either in a direct cause and effect manner or as a marker of processes that contribute to epithelial damage. Mucus metaplasia and smooth muscle hypertrophy seem to be features of asthma that are independent of eosinophilia.

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Priming of human basophils by low levels of anti-FcεRI α-chain mAb

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Chronic exposure to allergens is generally thought to be a risk factor for exacerbation of allergic diseases. However, the precise roles of very low levels of those antigens and the corresponding antibodies are largely unknown. In this study, we analyzed whether very low levels of anti-FcεRI monoclonal antibody (mAb) modulate the function of basophils.

Basophils were obtained from normal human peripheral blood. Semi-purified or highly purified basophil preparations were incubated with subthreshold concentrations (~1 ng/ml) of anti-FcεRI α-chain mAb (CRA-1). Surface activation marker CD69 expression, basophil migration and secretagogue-induced activation of basophils were analyzed.

The level of surface CD69 expression induced by IL-3 was enhanced in the presence of either low (1 ng/ml) or high (100 ng/ml) concentrations of CRA-1 mAb. The low level of this mAb was similarly or even more potent than the high level in that enhancement. Eotaxin-directed basophil migration was enhanced when 1 ng/ml of CRA-1 mAb, but not 1 ng/ml of control IgG2b, was included in the migration buffer. Histamine release triggered by a chemokine, MCP-1, was enhanced when cells were preincubated with CRA-1 mAb at 1 ng/ml for 1 hr or IL-3 at 300 pM for 15 min. Preincubation of basophils with both CRA-1 mAb and IL-3 further enhanced the cells' response to MCP-1. Preincubation of basophils with low concentrations, i.e., 1 ng/ml, of CRA-1 mAb enhanced LTC4 secretion triggered by FMLP. Human cultured mast cells showed slightly enhanced degranulation triggered by TPA when they were preincubated with CRA-1 mAb at 100 ng/ml for 1 hr. These results indicate that very low, even subthreshold, concentrations of anti-FcεRI mAb can prime basophils for enhanced motility and activation. These results may partly explain how and why chronic exposure to very low levels of antigen can affect the clinical manifestation of allergic diseases in which basophils are thought to play an effector role.

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Dipeptidyl peptidase I modulates the generation of active tryptase and chymase in human mast cells

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Background: The mast cell proteases tryptase and chymase are synthesized as inactive proenzymes. Dipeptidyl peptidase 1 (DPPI) can activate proforms of tryptase and chymase in cell free systems, but there has been conflicting evidence over the extent to which it fulfils this role within mast cells. We have examined the ability of DPPI to modulate the activation of tryptase and chymase in mast cells.

Methods: LAD-2 and HMC-1 mast cell lines were cultured with the DPPI inhibitor Gly-Phe-CHN_2 or with novel small molecule inhibitors of DPPI for up to 48h. Levels of active tryptase and chymase were measured in cell lysates and supernatants using chromogenic substrates before and following addition of exogenous DPPI. Quantities of tryptase, chymase and DPPI were also measured by ELISA, and Western blotting performed with DPPI-specific antibodies. Cells of the mast cell lines incubated with DPPI inhibitors were experimentally activated (using anti-IgE antibody or calcium ionophore A23187) or incubated without stimulus, and levels of tryptase, chymase or DPPI determined. Parallel studies were

conducted with mast cells dispersed from human lung, tonsil or skin.

Results: Culture of LAD-2 cells with DPP1 inhibitors resulted in significant decreases in both tryptase and chymase activity in cell lysates within 24h. Levels of immunoreactive tryptase and chymase were unaffected. Addition of DPPI restored the activity of these two mast cell proteases, suggesting that inhibition of DPPI had resulted in the accumulation of proforms of tryptase and chymase in cells. In HMC-1 cells (which are deficient in chymase) a reduction in tryptase activity was observed following addition of DPPI inhibitors. The degranulation of DPPI inhibitor-treated cells could be induced with ant-IgE or calcium ionophore, though release of tryptase or chymase (by measurement of activity or ELISA) was substantially reduced. On degranulation only the active forms of tryptase and chymase were released, though there was evidence for spontaneous release of the proforms for both of these enzymes consistent with constitutive release.

Conclusions: DPP1 may play a key role in controlling the activity of tryptase and chymase within mast cells and their release; and represents a promising target for therapeutic intervention in allergic disease.

ORAL ABSTRACT SESSION 5: T Cells and Immunoregulation

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Role of the new Th22 subset in allergy

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The impact of uncontrolled activation of Th2 cells in allergic sensitization and IL-4-dependent switch and production of IgE are well-established. While IgE-dependent mechanisms trigger recruitment and activation of mast cells, basophils and eosinophils, it has become apparent that Th17 cells are also involved in allergic inflammation. Here we describe a novel subset, the Th22 cells, that infiltrate the epidermis in allergic inflammatory skin disorders and are characterized by the secretion of IL-22 and TNF-α, but lack IFN-γ, IL-4 and IL-17. To determine whether these cells are truly a different subset, we cloned Th22 cells and demonstrate that they are characterized by stable secretion of IL-22. Similarly we could isolate stable Th1, Th2 and TH17 clones, which we compared on the full transcriptome level against the Th22 clones and demonstrate that they are statistically as separate from Th17 cells as Th1 from Th2 cells. The analysis also revealed characteristic Th22 products, which are not expressed by Th1, Th2 or Th17 cells, including transcription factors, remodeling genes like Fibroblast growth factors and angiogenesis/fibrosis chemokines. The functional relevance of Th22 cells was investigated in cultures where keratinocytes were exposed to Th22 supernatants. The full transcriptome analysis of these cultured revealed a specific, novel response profile that combines genes of the innate immune pathways (induction of TLR3, complement cascade and defensins) as well as recruitment (CXCL chemokines) and modulation (IL-7, IL-15) of adaptive immunity. These pro-inflammatory Th22-functions are dependant on IL-22 and TNF-α in a synergistic manner. Th22 supernatants enhanced rapid and complete wound healing in an in vitro injury model. In conclusion, human Th22 subset represents a separate T cell subset with distinct identity for gene expression and function, present within the epidermal layer of allergic, inflammatory skin diseases. We hypothesize that Th22 cells play an important role in the transition between acute allergic inflammation into chronic conditions, which are associated with excessive remodeling. Future interventions aiming on remodeling in chronic disorders may profit from Th22-subset directed therapies.

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Tonsils are organs of immunotolerance to develop allergen-specific immunotherapy approaches targeting dendritic cells and Treg cells

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Due to their strategic location palatine tonsils are constantly exposed to microbes and constitute the first contact point of the immune system to food or aeroallergens (before their degradation by digestive enzymes and highly acidic gastric secretion). The aim of this study was to investigate the potential role of human palatine tonsils in oral tolerance induction. Single-cell suspensions of tonsil mononuclear cells (TMCs) were isolated by mechanical disruption and fractionated by centrifugation on a density cushion. Purifed tonsil plasmacytoid DCs cells (TpDCs), myeloid DCs (TmDCs) and FOXP3+ Treg cells were phenotypic and functional characterized by flow cytometry and suppressive assays. Proliferation was evaluated by ^{3}H-thymidine incorporation or CFSE dilution experiments. Real-time quantitative PCR, quantification of cytokine levels in supernatants and intracellular staining experiments were performed. Tonsil biopsies were analyzed by confocal microscopy. Our results demonstrated that $CD4^{+}FOXP3^{+}$ Treg cells with suppressive activity and pDCs with the capacity to generate functional $CD4^{+}FOXP3^{+}$ Treg cells constitute major T and DC compartments in human palatine tonsils. $FOXP3^{+}$ Treg cells co-localize with pDCs and proliferate *in vivo* in the T cell areas of human palatine tonsils in close contact to crypt epithelial cells. In addition, we showed that tonsils from atopic individuals resemble the classical features of allergic diseases at the T cell level and cytokine signature. We also showed that allergen-specific T cell responses can be mounted in tonsils and that TLR8, but not TLR7 and TLR9-triggering as innate inflammatory factors break

allergen-specific tolerance in human palatine tonsils. In conclusion, the present study demonstrated that human palatine tonsils represent an immunotolerance inducing lymphoid tissue with the capacity to generate $CD4^+FOXP3^+$ Treg cells that might well be clinically exploited to develop more efficient and safer ways of allergen-specific immunotherapy for the treatment of allergic diseases.

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Changes in regulatory T cells activity during early childhood: Novel studies using thymic tissue

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Background: Characterisation of regulatory immune pathways is a research priority to define both the pathogenesis and potential therapeutic strategies for allergic disease.

Aim: The thymus offers a novel opportunity to document the maturation of these pathways, which are otherwise difficult to study in the only small volumes of peripheral blood available from young infants.

Methods: Thymic tissue was collected during cardiac surgery in otherwise healthy children aged 1 week – 12 years of age, with no evidence of immune defects. Samples were processed into single-cell suspension of thymocytes by passing the tissue through a 70μm nylon sieve. These were used for flow cytometry, cell culture work and mRNA extraction.

Results: Age-related changes in the cytokine milieu in the thymus parallel changes in peripheral immune function. Specifically, the thymic microenvironment is similarly "Th2-skewed" in the early postnatal period (with higher IL-5 and IL-13 cytokine production), and this undergoes age-related suppression as Th1 (IFNγ) production increases. This was seen with both constitutive and stimulated thymocyte cytokine production. IL-10 responses also increased with age. CD4+CD25+CD127lo/-FOXP3+ T regulatory (Treg) cells were readily identified and comprised ~5% of the total proportion of thymocytes in neonates and this proportion increased with age in parallel to an age-related increase in FOXP3 mRNA expression. The percentage of Ki67+ cells was examined as a measure of the proliferative expansion of CD4+CD25+CD127lo/-Treg, presumably mediated by a combination of self- and environmental antigens. We observed greater Treg expansion in the older (25.4%) compared with younger children (17.8%), suggesting that there may be increased thymic Treg expansion with age.

Conclusions: These data suggest that the developmental changes in the thymus parallel recognized changes in peripheral blood responses, and support our hypothesis that there are also age-related changes in regulatory function. Similar technologies will now be used to compare children with allergic and nonallergic phenotypes.

Funding: NHMRC Australia

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Ontogeny of toll-like receptor expression and function in allergic and non-allergic children: The first 5 years

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Background: Reduced microbial exposure in early life has become a leading candidate to explain the escalating rate of allergic disease. It is hypothesised that alteration in microbial responses through differences in toll-like receptors (TLR) function may predispose to the development of allergic disease. To our knowledge this is the first longitudinal study to document expression and function of TLRs in children and to explore the association between early TLR microbial recognition and prospective allergy development.

Methods: Cord and peripheral blood mononuclear cells (MC) were collected at birth, 1, 2.5 and 5 years of age. Clinical evaluations occurred at each postnatal visit, and immune function was compared in allergic children (n=35) who had evidence of sensitisation and doctor-diagnosed allergic disease to nonallergic children (n=35) who were never sensitised or had evidence of allergy. Innate and adaptive immune function were assessed by stimulating MC with TLR ligands, allergens or mitogens. Cytokines were measured from the supernatant using Luminex. Innate and regulatory gene expression was measured from cell pellets with real-time PCR. APC expression of TLRs was examined with FACS.

Results: In non-allergic children, innate TLR function increased ($P=0.003$) during the first 2.5 years of life and plateaued thereafter. This was seen at both mRNA and protein level. These children also showed age-dependent maturation of IFNγ production in response to mitogens. Conversely, allergic group, which had higher IL-10, TNFα and IL-6 TLR responses at birth compared to nonallergic children (all $P<0.01$), showed down-regulation of these responses after the first year. In keeping with other studies, allergic children had low IFNγ responses at birth and this did not increase until after 2.5 years of age. In contrast, allergic children displayed age-dependent increase in allergen-specific Th2 responses. By age 5, allergic children had significantly reduced innate function compared to control children ($p=0.01$). Children who go on to develop allergic disease have higher proportion of TLR2+ pDCs at birth, compared to children who remain non-allergic ($P<0.001$).

Conclusions: This project provides the first longitudinal data describing the ontogeny of key microbial pattern recognition receptors in early childhood and suggests the importance of pDCs in infants who go on to develop allergic disease.

Funding: NHMRC Australia

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IL-13 attenuates IL-17A production in an IL-10 dependent manner in mouse and human Th17 cells

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Introduction: Patients with inflammatory bowel disease (IBD) often have increased numbers of hTryptase-β^+ mast cells (MCs) in their colons, but the significance of this observation has not been elucidated. Mouse mast cell protease-6 (mMCP-6) is the ortholog of hTryptase-β, and we previously noted that this tryptase has beneficial roles in bacterial and helminth infections but adverse roles in two experimental models of arthritis. Due to the latter findings, we hypothesized that mMCP-6 might have prominent pro-inflammatory and/or connective tissue remodeling roles in experimental colitis.

Methods: Wild-type (WT) and mMCP-6-null C57BL/6 (B6) mice were evaluated in a colitis model in which the animals were given 1.5% dextran sodium sulfate (DSS) in their drinking water for 7 days. The extent of weight loss was assessed, as well as the individual histopathology scores of their colons. RNA also was isolated, and the levels of the transcripts that encode all known mouse proteins in the diseased colons were assessed by microarray analysis. Confirmatory real-time qPCR, ELISA, and/or immunohistochemistry analyses were used to validate and extend the microarray data.

Results: DSS-treated WT mice lost 7.0% ± 1.0 (n = 27) of their weight, whereas similarly treated mMCP-6-null mice lost no weight. Using a 20-point histopathology scoring system, WT mice scored a much more severe pathology index relative to similarly-treated mMCP-6-null mice. GeneChip and real-time qPCR analyses revealed that the transcripts that encode numerous chemokines that preferentially attract neutrophils (e.g., Cxcl1 and Cxcl2), matrix metalloproteinases that participate in the remodeling of extracellular matrices (e.g., MMP-3, MMP-9, and MMP-13), and cytokines that promote inflammation (e.g., IL-1β and IL-6) were all markedly increased in the colons of DSS-treated WT mice relative to untreated WT mice and DSS-treated mMCP-6-null mice. ELISA and/or immunohistochemistry analyses revealed changes in the corresponding protein levels of many of these differentially expressed transcripts.

Conclusion: mMCP-6 is essential in DSS-induced colitis, and this MC-restricted tryptase acts upstream of many of the factors implicated in IBD in humans.

ORAL ABSTRACT SESSION 6: Regulatory Cells and Memory Stem Cells

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Identification of CD8+ memory stem cells guides to novel strategies for adoptive T cell therapy

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It is well accepted that $CD8^+$ T cells play a pivotal role in providing protection against infection with intracellular pathogens and some tumors. In many cases protective immunity is maintained for long periods of time (immunological memory). Over the past years, it has become evident that in order to fulfill these multiple tasks, distinct subsets of effector and memory T cells have to be generated. Until today, however, only little is known about the underlying mechanisms of subset differentiation and the timing of lineage fate decisions. In this context, it is of special importance to determine at which level of clonal expansion functional and phenotypical heterogeneity is achieved. Different models for T cell subset diversification have been proposed; these differ mainly in the time point during priming and clonal expansion (prior, during, or beyond the first cell division) when differentiation programs are induced. Recently developed single-cell adoptive transfer technology has allowed us to demonstrate that individual precursor cells (naïve and some memory T cells) still bear the full plasticity to develop into a plethora different T cell subsets. This observation targets the shaping of T cell subset differentiation towards factors that are still operative beyond the first cell division and point at the existence of so-called 'memory stem cells' within the memory T cell pool. These findings have important implications for vaccine development, as the modulation of differentiation patterns towards distinct subsets could become a powerful strategy to enhance the efficacy and quality of vaccines. Furthermore, our findings indicate that adoptive T cell immunotherapies might be possible to perform with very low cell numbers, which could facilitate the transfer of this promising approach to a variety of different clinical applications.

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Innate production of Th2 cytokines by "natural helper cells"

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Innate immune responses are important in combating various microbes during the early phases of infection. Natural killer (NK) cells are innate lymphocytes that, unlike T and B lymphocytes, do not express antigen receptors but rapidly exhibit cytotoxic activities against virus infected cells and produce various cytokines. We recently discovered a new type of innate lymphocyte present in a novel lymphoid structure associated with adipose tissues in the peritoneal cavity. These cells do not express lineage (Lin: CD3, CD4, CD8α, TCRβ, TCRδ, CD5, CD19, B220, NK1.1, TER119, Gr-1, Mac-1, CD11c, FceRIα) markers but express c-Kit, Sca-1, IL-7R and IL-33R. Similar lymphoid clusters were found in both human and mouse mesentery and we term this tissue "FALC" for fat-associated lymphoid cluster. FALC $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ cells are distinct from lymphoid progenitors and lymphoid tissue inducer (LTi) cells. These cells proliferate in response to IL-2 and produce large amounts of Th2 cytokines such as IL-5, IL-6 and IL-13. IL-5 and IL-6 regulate B cell antibody production and self-renewal of B1 cells. Indeed, FALC $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ cells support the self-renewal of B1 cells and enhance IgA production. IL-5 and IL-13 mediate allergic inflammation and protection against helminth infection. Upon helminth infection and in response to IL-33, FALC $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ cells produce large amounts of IL-13, which leads to goblet cell hyperplasia, a critical step for helminth expulsion. In mice devoid of FALC $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ cells such goblet cell hyperplasia was not induced. Thus, FALC $Lin^-c\text{-}Kit^+Sca\text{-}1^+$ cells are Th2-type innate lymphocytes playing an important role in the early phase of helminth infection and we propose that these cells be called "natural helper cells".

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Human natural killer cell subsets: Their potential immunoregulatory roles

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The immune system has a variety of regulatory/suppressive processes, which are decisive for the healthy and allergic immune response to allergens. Similar to Th1 and Th2 cells, human NK cells cultured in the presence of IL-12 or IL-4 differentiate into cell populations with distinct patterns of cytokine secretion. The *in vivo* existence of human NK cell subsets similar to Th1 and Th2 cells was demonstrated in freshly isolated IFN-γ-secreting and IFN-γ-nonsecreting NK cells. The IFN-γ-secreting NK subset showed a typical cytokine pattern with predominant expression of IFN-γ, but almost no IL-4, IL-5 and IL-13. In contrast, IFN-γ-nonsecreting NK cells mainly produce IL-13 and contribute to IgE production by B cells. While production and role of TGF-β and IL-10 in human peripheral blood NK cells have been demonstrated, regulatory subsets of NK cells have yet to be fully characterized. To investigate the existence of regulatory NK cells in humans, NK cell subsets were

purified and characterized according to their IL-10 secretion property. IL-10-secreting NK cells expressed CD16 and CD56, activation markers CD25, CD69, CD49d and killer activatory CD94, CD161 and killer inhibitory receptors, CD158a, NKAT2 on their surface. Freshly purified IL-10-secreting NK cells expressed up to 40- fold increase in IL-10, but not in the FoxP3 and TGF-b mRNAs. PHA and IL-2 stimulation as well as vitamine D3/dexamethasone and anti-CD2/CD16mAbs are demonstrated to induce IL-10 expression in NK cells. Frequency of IL-10-secreting NK cells was significantly low (2-6 %) compared to IFN-γ-secreting NK cells (61-89 %). As previously observed in IFN-γ^+ and IFN-γ^- NK subsets, IL-10^+ and IL-10^- NK cells did not show any difference in their natural cytotoxicity to K562 cells. The effect of IL-10^+ NK cells on antigen-specific T cell proliferation was examined in bee venom major allergen, phospholipase A_2- or purified protein derivative of *M. bovis*-induced T proliferation. IL-10^+ NK cells significantly suppressed both allergen/antigen-induced T cell proliferation, particularly due to secreted IL-10 as demonstrated by anti-IL-10 receptor blocking mAb. For comparison IFN-γ-secreting NK cells did not show any suppression. These results demonstrate that circulating NK cells retain effector subsets in humans with distinct cytokine profiles and may display different inflammatory properties.

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Allergen-specific IL-17^+IL-4^+CD4^+ T cells circulate in patients with Type I allergy

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Recently, a new subset of CD4^+ T cells has been identified producing the key cytokine IL-17A. So-called Th17 cells can be driven to IFN-γ secretion. However, their potential to produce IL-4 and their involvement in allergic diseases is less clear. We isolated two T cell clones, SD266 and SD334, specific for the major birch pollen allergen Bet v 1 from the peripheral blood of a birch pollen-allergic individual under exactly the same culture conditions. Both clones reacted to the immunodominant epitope of Bet v 1. Sequencing of their T cell receptor α and β chains demonstrated that both clones were identical. Interestingly, they markedly differed in their cytokine responses to Bet v 1. SD266 synthesized high amounts of IL-2, IL-4, IL-17 and IFN-γ whereas SD334 did not. Both clones secreted varying amounts of IL-6, IL-10 and TNF-α while SD334 also produced GM-CSF. The fact that SD266 produced enormous amounts of IL-4 and IL-17 provided first evidence for the existence of IL-4^+IL-17^+ T cells specific to allergens causing Type I allergy. To substantiate this evidence, the cytokine profiles of additional 38 T cell clones specific to major allergens in pollen of ragweed, mugwort and timothy grass as well as in hazelnuts were analysed. Nine clones produced IL-17 in response to allergen. All IL-17^+ allergen-specific clones concomitantly synthesized IL-4 and 7/9 clones secreted IFN-γ, IL-10 or IL-2. These data confirmed that allergen-specific IL-4^+IL-17^+CD4^+ T cells circulate in patients with Type I allergy. In our opinion, the identification of IL-4^+IL-17^+ T cells adds another phenotype to the highly heterogeneous subset of human allergen-specific Th2 cells. The observation that an allergen-specific clone can exist as IL-17^+ Th2-like cell and IL-17^- Th0-like cell is intriguing. This study demonstrates the *in vivo* plasticity of allergen-specific Th cells and reinforces the notion that IL-17 may play a role in IgE-mediated disorders.

ORAL ABSTRACT SESSION 7: Dendritic Cells and Macrophages

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Inhibition of Th2 adaptive immune responses and pulmonary inflammation by leukocyte Ig-like receptor B4 on dendritic cells

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We previously established that the inhibitory receptor LILRB4 mitigates LPS-induced, neutrophil-dependent pathologic effector mechanisms in inflammation. We now report that LILRB4 on dendritic cells (DCs) counterregulates development of an adaptive Th2 immune response and ensuing inflammation in a model of allergic pulmonary inflammation initiated by inhalation sensitization with OVA and LPS followed by airway challenge with OVA. We found that *Lilrb4*$^{-/-}$ mice had significantly exacerbated eosinophilic pulmonary inflammation as assessed in bronchoalveolar lavage and lung tissue, as well as elevated levels of OVA-specific IgE and Th2 cytokines produced by OVA-restimulated lymph node cells. LILRB4 was preferentially expressed on MHC class II^{high}CD86^{high} OVA-bearing DCs in lung-draining lymph nodes after sensitization or challenge. Moreover, the lymph nodes of *Lilrb4*$^{-/-}$ mice had significantly more of these mature DCs after challenge with OVA, which was accompanied by significantly more IL-4-producing lymphocytes, compared with *Lilrb4*$^{+/+}$ mice. Sensitization of naïve *Lilrb4*$^{+/+}$ mice by transfer of OVA-LPS-pulsed *Lilrb4*$^{-/-}$ bone marrow-derived DCs (BMDCs) was sufficient to confer exacerbated allergic lung pathology upon challenge with OVA, compared with mice that received *Lilrb4*$^{+/+}$ BMDCs. Our findings establish that maturation and migration of pulmonary DCs to lymph nodes in response to Ag and an innate immune stimulus is associated with upregulated expression of LILRB4. In addition, this receptor attenuates the number of these mature DCs and attendant IL-4-producing lymphocytes in the lymph nodes, and accordingly, the ability of DCs to elicit pathologic Th2 pulmonary inflammation.

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Histamine modulates dendritic cell toll-like receptor activation via the histamine receptor 2

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Introduction: Dendritic cells (DC) are potent antigen presenting cells that are present at all mucosal surfaces and are central players in initiating and regulating adaptive immune responses. DC activation, maturation and polarization are largely influenced by local factors within their micro-environment such as microbial components, cytokines and metabolic products. *In vivo*, multiple DC pattern recognition receptors are simultaneously activated and the co-operation or competition between the resultant signaling cascades is not well understood. Histamine is an additional factor present within the DC micro-environment of allergic individuals and has received little attention for its effects on human DC activation.

Aim: To determine the impact of histamine co-stimulation on dendritic cell activation by microbial ligands.

Methods: The human THP-1 monocyte cell line (incorporating a NF-κB-SEAP reporter) and primary human myeloid DCs were stimulated with LPS (TLR-4 agonist), Pam3Cys (TLR-2 agonist),

flagellin (TLR-5 agonist), *Pseudomonas aeruginosa* (gram negative bacterium) or *Streptococcus pyogenes* (gram positive organism) following histamine co-incubation. Multiplex cytokine analysis was performed using the Luminex system while cAMP levels were measured by ELISA. CREB phosphorylation and dendritic cell co-stimulatory molecule expression was quantified using flow cytometry.

Results: Histamine suppressed NF-κB induction in response to TLR-2, TLR-4, TLR-5 agonists and bacterial stimulation in a dose-dependent manner. In addition, TNF-α and IL-12 production was significantly suppressed while IL-10, G-CSF and cAMP production and CREB phosphorylation were significantly enhanced. Upregulation of CD80, CD83, CD86 and HLA-DR cell surface expression in response to LPS was not altered by histamine co-stimulation. Each histamine receptor (H1R-H4R) was independently blocked and only Cimetidine (H2R antagonist) blocked the histamine suppressive effect. NF-κB and TNF-α suppression could be mimicked using Dimaprit alone (H2R selective agonist).

Conclusion: These results demonstrate that histamine exerts a polarising effect on dendritic cells responding to microbial stimuli via the H2R which could have a critical impact on local immunoregulation within the mucosa, in particular in patients with allergy and asthma.

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A subpopulation of neutrophils induces FcεRI on mouse lung conventional dendritic cells through a CD11b dependent process

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Severe paramyxoviral infections have been shown to greatly increase the risk of atopic disease. The mechanisms underlying the translation of viral infection into allergy are not well known. Using a mouse model, we have shown that during a severe paramyxoviral (Sendai virus, SeV) infection lung conventional dendritic cells (cDC) express FcεRI, and that this receptor is necessary for the development of post-viral atopic disease. Although we have previously shown that induction of FcεRI is IgE independent and requires expression of the type I IFN receptor (IFNAR), the actual mechanism is not known. We hypothesized that neutrophils (PMN) may be responsible for induction of FcεRI on lung cDC. We treated C57BL6 (WT) mice with either an anti-Ly-6G or control monoclonal antibody (mAb) every other day starting one day prior to SeV infection and found that anti-Ly-6G treatment prevented FcεRI expression on cDC [1.6±0.0 (anti-Ly-6G) versus 11.1±0.2 (control IgG) fold MFI FcεRI expression, p=0.0005]. Depleting either of the other 2 Gr-1 expressing cells (NK cells or plasmacytoid dendritic cells) failed to reduce FcεRI expression on cDC. PMN isolated from bronchoalveolar lavage (BAL) of day 3 p.i. SeV WT but not IFNAR deficient (*IFNAR*$^{-/-}$) mice were capable of inducing FcεRI expression on naïve lung cDC after 48 hours of co-culture [3.7±0.3 (WT) versus 1.4±0.1 (*IFNAR*$^{-/-}$) fold MFI FcεRI expression, p=0.0004]. PMN from BAL of SeV infected WT mice contained a subpopulation that expressed CD49d, and only these CD49d^{+} PMNs were capable of inducing FcεRI expression on cDC in our co-culture system [9.7±0.1 versus 1.0±0.0 fold MFI FcεRI expression, p=0.0003]. The CD49d^{+} PMN were markedly reduced in BAL from SeV infected *IFNAR*$^{-/-}$ mice [13.7±2.0% of PMN in WT versus 4.3±0.3% in *IFNAR*$^{-/-}$, p=0.002]. Finally, we found addition of a CD11b blocking mAb to the PMN prior to co-culture prevented induction of FcεRI on the cDC [2.6±0.2 (anti-CD11b) versus 12.3±1.6 (control IgG) fold MFI FcεRI expression, p=0.03]. We conclude that induction of FcεRI on lung cDC depends upon CD11b function on CD49d^{+} PMN; these studies highlight the importance of PMN-cDC axis in translating viral into atopic disease.

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Human dermal fibroblasts support the differentiation of IL-17 producing T-cells via up-regulation of IL-23 production by dendritic cells

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To trigger an effective T cell-mediated immune response in the skin, upon antigen contact dendritic cells (DC) migrate into locally-draining lymph nodes where they present antigen to naive T cells and induce activation and differentiation of T cells (TC). During their migration to secondary lymphoid organs, DC travel through the stromal microenvironment comprised of the extracellular matrix and stromal cells such as fibroblasts, macrophages and endothelial cells. Little is known about the interaction of DC with the stromal microenvironment. Recently, we have shown that DC interact with dermal fibroblasts in vivo and in vitro in inflamed skin.

To study the effects of this interaction, monocyte-derived DC were partially matured by adding Lipopolysaccharide (DC-LPS) to imitate antigen contact in inflamed skin. Following, they were cocultured with dermal fibroblasts for 24h. We could demonstrate that LPS-stimulated DC activated fibroblasts via tumour necrosis factor (TNF) α and IL-1β. Consequently, activated fibroblasts produced prostaglandin (PG) E_2 which in turn increased interleukin-23 (IL-23) production by DC compared to LPS-stimulated DC. Since IL-23 is an important factor in the differentiation of TH17 cells, we stimulated CD3^{+} T cells or memory T cells with either supernatants of DC-LPS-fibroblast-co-culture or DC-LPS in the presence of anti-CD3/CD28-beads. Indeed, supernatants of DC-LPS-fibroblast-co-culture significantly increased IL-17 production of CD3+ T cells or memory T cells compared to T cells stimulated with DC-LPS supernatant alone.

In brief, we were able to demonstrate that dermal fibroblasts regulate cytokine production of DC and thus are involved in the control of TC differentiation.

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Synergistic effect of secreted phospholipases A_2 and adenosine on the induction vascular endothelial growth factors in human lung macrophages

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Angiogenesis mediated by vascular endothelial growth factors (VEGFs) is a main feature of chronic inflammation and tumors. Tissue macrophages play a pivotal role in both pathological

processes. We have previously shown that secreted phospholipases A_2 ($sPLA_2$s) induce cytokine/chemokine production from human macrophages by a receptor-mediated mechanism. Adenosine is a purine nucleoside known to promote the proangiogenic activity of macrophages. Since $sPLA_2$s and adenosine are both produced at sites of inflammation and tumor growth, we investigated the effect of these mediators on the production of VEGFs from primary human macrophages (HLM) purified from the lung tissue. VEGF expression and release was evaluated by RT-PCR, western blot, and ELISA in HLM either unstimulated or activated by $sPLA_2$s and adenosine. Angiogenic activity of macrophage supernatants was determined by chick embryo chorioallantoic membrane (CAM) assay. HLM express VEGF-A, VEGF-B, VEGF-C, and VEGF-D, but not PlGF, at both mRNA and protein level. Two human $sPLA_2$s (group IIA: hGIIA, and group X: hGX) induced a concentration-dependent release of VEGF-A (unstimulated: 59±16 pg/mg of protein, hGIIA: 376±42, hGX: 740±32) and VEGF-C (unstimulated: 111±75 pg/mg of protein, hGIIA: 342±61, hGX: 697±67). In addition, hGX induced gene transcription of both VEGF-A (12.49±1.06 fold increase *vs* unstimulated) and VEGF-C (8.80±1.35). Enzymatically-inactive $sPLA_2$s were as effective as the active enzymes in inducing VEGF production. Preincubation (37°C, 30 min) of hGX with Me-Indoxam and RO092906A, two compounds blocking receptor-mediated effects of $sPLA_2$s, inhibited the release of VEGF-A and VEGF-C. Supernatants of hGX-activated HLM induced an angiogenic response in the CAMs which was inhibited by Me-Indoxam. Adenosine analogs that bind the receptor A_{2A} (NECA and CGS-21680) induced the release of VEGF-A only at 10 μM. Stimulation of HLM with hGX in the presence of NECA and CGS-21680 induced a synergistic increase of VEGF-A release and completely blocked TNF-α production. Our results demonstrate that human macrophages express several VEGFs. $sPLA_2$s induce the production of VEGF-A and VEGF-C from human macrophages by a receptor-mediated mechanism. Adenosine modulates $sPLA_2$-induced activation of macrophages by inducing a functional switch of these cells towards a proangiogenic phenotype. Thus, $sPLA_2$s and adenosine cooperate to regulate inflammatory and/or neoplastic angiogenesis.

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Group V secretory phospholipase A_2 modulates phagosome maturation and regulates the innate immune response against *Candida albicans*

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Phospholipases A2 (PLA2) are a family of enzymes that hydrolyze the ester bound of membrane phospholipids to generate free fatty acid and lysophospholipids. When the free fatty acid is arachidonic acid an enzymatic cascade generates eicosanoids. By using a gene knock-out strategy, we reported that group V secretory PLA2 (sPLA2) regulates eicosanoid generation and phagocytosis of zymosan by mouse peritoneal macrophages. The aim of this study was to indentify the mechanism by which group V sPLA2 regulates phagocytosis and to determine its role in the innate immune response against Candida albicans. Here we report that during phagocytosis of zymosan, macrophages lacking group V sPLA2 had a significant reduction in phagosome maturation at early time points. We also found that the binding of zymosan was equally efficient in both wild type and group V sPLA2-null macrophages. Importantly, macrophages lacking group V sPLA2 had delays in phagocytosis, phagosome maturation and killing of Candida albicans. However, cytokine production and eicosanoid generation were unaffected by the lack of group V sPLA2. Furthermore, we investigated the role of group V sPLA2 in a murine model of systemic candidiasis. We found that mice lacking group V sPLA2 had an increased fungal burden in the kidney, liver and spleen at day 7 post-infection and increased mortality. These data demonstrate that group V sPLA2 regulates phagocytosis through a mechanism that is likely dependent on phagolysosome fusion and that it contributes to the innate immune response against C. albicans by regulating phagocytosis and killing.

ORAL ABSTRACT SESSION 8: Pathophysiology of Allergic and Inflammatory Diseases

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Localization of the calcitonin-gene-related peptide-associated receptor activity modifying protein 1 (RAMP1) to airway epithelial cells and its dysregulation in asthma

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Background: Enhanced epithelial cell expression of the calcitonin-gene-related peptide (CGRP) is a feature of allergen peptide-induced late-phase asthmatic reactions. The receptor activity modifying protein 1 (RAMP1**),** a single-transmembrane-domain protein, functions as the CGRP receptor in conjunction with the calcitonin-receptor-like receptor. Here we describe chemokine-induced synthesis and release of CGRP in vitro and the expression of RAMP1 by airway epithelial cells from asthmatics and normal subjects. We have also studied receptor-mediated functional effects of CGRP on airway cell lines.

Methods: Bronchial biopsies from peptide–induced late asthmatics responders and non-responders, and, non- asthmatic controls were examined by immunohistochemistry and *in situ* hybidization. BEAS-2B and A549cells lines were studied by an immunoflouresence assay and proteins measured by ELISA.

Findings: In order to demonstrate that CGRP immunoreactivity in asthmatic epithelial cells had functional significance we showed that several inflammatory markers (CCL17, IL-13 and TNF-alpha) all produced between a 10^3 and 10^5 increase in CGRP mRNA and protein in A549 and BEAS-2B cells in a time and dose-dependent fashion. These cell lines also expressed functional CGRP receptors. Thus RAMP1 was constitutively expressed on both A549 and BEAS-2B cells and incubation with CGRP resulted in RAMP1 internalisation, and secretion of IL-6. Both of these events were blocked by the CGRP antagonist CGRP8-37, suggesting biological activity of CGRP in bronchial epithelial cells is mediated by the CGRP receptor. There was also clear RAMP-1 immunoreactivity and mRNA expression in the epithelium and smooth muscle in normal airways. However in asthma baseline expression of epithelial (but not smooth muscle) mRNA and protein for RAMP1 was significantly decreased compared to normals ($p=0.0007$ and $p=0.0002$ respectively). In addition peptide allergen challenge produced receptor down-regulation in responders compared to non-responders ($p=0.027$).

Conclusions: Airway epithelial cells synthesise CGRP in response to several inflammatory mediators, express RAMP1 and respond to CGRP with cytokine production. There is an apparent dysregulation of RAMP1 in asthmatic epithelium suggesting continuous stimulation of pathways involving CGRP.

Abstracts

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Tryptase-dependent experimental colitis

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Introduction: Patients with inflammatory bowel disease (IBD) often have increased numbers of hTryptase-β^+ mast cells (MCs) in their colons, but the significance of this observation has not been elucidated. Mouse mast cell protease-6 (mMCP-6) is the ortholog of hTryptase-β, and we previously noted that this tryptase has beneficial roles in bacterial and helminth infections but adverse roles in two experimental models of arthritis. Due to the latter findings, we hypothesized that mMCP-6 might have prominent pro-inflammatory and/or connective tissue remodeling roles in experimental colitis.

Methods: Wild-type (WT) and mMCP-6-null C57BL/6 (B6) mice were evaluated in a colitis model in which the animals were given 1.5% dextran sodium sulfate (DSS) in their drinking water for 7 days. The extent of weight loss was assessed, as well as the individual histopathology scores of their colons. RNA also was isolated, and the levels of the transcripts that encode all known mouse proteins in the diseased colons were assessed by microarray analysis. Confirmatory real-time qPCR, ELISA, and/or immunohistochemistry analyses were used to validate and extend the microarray data.

Results: DSS-treated WT mice lost 7.0% ± 1.0 (n = 27) of their weight, whereas similarly treated mMCP-6-null mice lost no weight. Using a 20-point histopathology scoring system, WT mice scored a much more severe pathology index relative to similarly-treated mMCP-6-null mice. GeneChip and real-time qPCR analyses revealed that the transcripts that encode numerous chemokines that preferentially attract neutrophils (e.g., Cxcl1 and Cxcl2), matrix metalloproteinases that participate in the remodeling of extracellular matrices (e.g., MMP-3, MMP-9, and MMP-13), and cytokines that promote inflammation (e.g., IL-1β and IL-6) were all markedly increased in the colons of DSS-treated WT mice relative to untreated WT mice and DSS-treated mMCP-6-null mice. ELISA and/or immunohistochemistry analyses revealed changes in the corresponding protein levels of many of these differentially expressed transcripts.

Conclusion: mMCP-6 is essential in DSS-induced colitis, and this MC-restricted tryptase acts upstream of many of the factors implicated in IBD in humans.

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Critical signaling nodes in mast cell proliferative and activation disorders

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Our laboratory is investigating the hypothesis that aberrant mast cell (MC) signaling underlies two recognized MC disorders: systemic mastocytosis (SM) and monoclonal MC activation syndrome (MMAS); and a third proposed disorder: MC activation syndrome (MCAS). SM is diagnosed on the basis of one major and one minor criterion, or three minor criteria. The major criterion is aggregates of MCs in marrow; the minor criteria are a serum tryptase > 20ng/ml, spindle shaped MCs within tissues, CD2 and/or CD25$^+$ MCs, and presence of the KIT D816V mutation. MMAS patients are diagnosed when presenting with anaphylaxis and two minor criteria. MCAS is diagnosed where primary and secondary disorders of MC activation are eliminated and where there are episodic symptoms consistent with MC mediator release, a decrease in symptoms with anti-mediator therapy, and an elevation in a biologic marker of MC activation. In a clinical study, to date, of 10 patients with anaphylaxis without demonstrable cause, 2 met the criteria for SM and 8 for MMAS. MC activation is regulated by a signaling network activated upon FcεRI aggregation. Our studies, and those of others, have revealed that these events are upregulated by coactivation of other receptors, for example KIT and G protein-coupled receptors such as the PGE_2-EP3 receptor. Thus, mutations and polymorphisms in these receptors and regulatory proteins may contribute to the above disorders. Based on studies in genetically modified mice, over 30 signaling molecules downstream of surface receptors have been implicated in abnormal MC activation. We have identified several such critical signaling nodes. Using approaches including gene knock-down in human and mouse MCs, we determined that LAT2, PI3K and Btk, are not only critical for optimal FcεRI-dependent MC activation but also for the ability of KIT to enhance this response. Of note is that D816V KIT enhances LAT2 phosphorylation, providing a link between SM and enhanced activation. The PI3K/Btk amplification axis was also critical for synergistically enhanced FcεRI/Kit- or FcεRI/EP3-mediated chemotaxis. Thus, the amplification signaling nodes that we have identified may not only be critical for anaphylactic responses, but also for homing MCs to lesions associated with disease.

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Regulation of tight junctions in human airway epithelium by T regulatory cells and their cytokines

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In chronic allergic inflammation, immune system cells and their cytokines interact with resident tissue cells, which leads to a series of tissue events after the activation, and proinflammatory cytokine and chemokine release from both sides. Some of these events seem to be part of the immune pathology, such as basement membrane thickening, epithelial death, desquamation and spongiosis, however, they also act as mechanisms that control the severity of tissue inflammation. Tight junctions (TJs) are the most apical component of the intercellular junctions complex within the epithelium. They form the barrier for diffusion of molecules, an access of external agents and cell migration between the tissue and lumen of the respiratory, alimentary and genital tracts. The modulation of epithelial barrier by the inflammatory processes and the immune system represents a potential mechanism for the treatment of chronic inflammatory diseases, such as asthma. We aimed to study the regulation of bronchial epithelium integrity by the immune system and external compounds. T regulatory cells and one of their recently demonstrated key cytokines, IL-35, decreased transepithelial resistance (TER) and induced stratification of membranes of two adjacent cells of human primary bronchial epithelial cells, suggesting the opening of tight junctions without affecting occludin, claudin-1, claudin-4 and ZO-1 mRNA expression. For controls,

THP1 monocyte cell line, when co-cultured with ALI, strongly decreased TER and slightly up-regulated occludin, ZO-1 and ZO-2 mRNA levels. Apical administration of AT-1002 peptide with high zonula occludens toxin (ZOT) activity naturally secreted by *Vibrio cholerae,* decreased epithelium integrity in a reversible manner. In contrast,, apical triggering of toll-like receptor 9 by CpG ODN increased TER, without affecting the mRNA expression of TJ proteins, suggesting the defensive enhancement of epithelium integrity against infectious agents. In conclusion these data demonstrate the complex and dynamic "open-close regulation" of the airway epithelium barrier by the immune system

ORAL ABSTRACT SESSION 9: Pathophysiology of Allergic Diseases

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Dysregulated innate and adaptive immune responses within the airways in severe asthma and the relevance of bacteria to disease persistence

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Treatment-resistant severe asthma has been found to have an altered T cell phenotypic profile within endobronchial mucosal biopsy samples as compared to that in mild asthma and healthy controls, with significantly enhanced gene expression for the transcription factors T-BET (T_H1) and RORC (T_H17) as well as, compared to the non-asthmatics, significantly increased GATA-3 (T_H2). This dysregulated T cell profile was matched by respectively enhanced cytokine gene expression (IFN-gamma, IL-17, TNF-alpha and IL-5) as well as evidence of a reduced regulatory T cell profile (Foxp3 and TGF-β).

To investigate the relevance of altered airway bacterial colonisation to this mucosal T cell profile, the culture-independent molecular microbiological approach of terminal-restriction fragment length polymorphism (T-RFLP), in conjunction with 16S rRNA gene clone analysis, was applied to investigate the microbiome in sputum and BAL samples from the same subject groups. Greater dominance of potentially pathogenic bacteria was significantly evident in severe asthma. Within severe asthma, dominant airway colonisation with *Haemophilus sp., Streptococcus sp.,* or *Moraxella catarrhalis* was associated with a higher neutrophil differential cell count (Median (IQR) 80% (67-83) vs 43% (29-67), p=0.001) and their total abundance positively correlated with the neutrophil differential cell count (p=0.037). There was a significant and positive correlation between sputum IL-8 and sputum neutrophil counts and the total abundance of these organisms (p<0.001). Within BAL in asthma there was also evidence of *Staphylococcus aureus* colonisation and FACS analysis revealed that the detection of *staphylococci* in asthma was associated with a skewed Vβ T cell repertoire (Vβ2, 5.1, 5.3 and 13.1) on CD4 but not CD8 T cells, consistent with staphylococcal enterotoxin (SE) related superantigenic stimulation. SEs may also act as nominal antigens and measurement of specific IgE against an SE mix (SEA, SEC and TSST-1) as well as SEB in severe asthma revealed significantly greater positivity and significantly higher serum levels than in mild asthma and healthy controls. There was also evidence of specific IgE against non-typeable *haemophilus influenzae*.

It is thus likely that an altered airway bacterial colonisation is an important determinant of disease persistence in treatment-resistant severe asthma. This appreciation may lead to different avenues for therapy.

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Interactions between inflammatory pathways triggered by host responses to respiratory viruses and aeroallergens in the aetiology and pathogenesis of atopic asthma: Acute severe asthma exacerbations as a paradigm

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We and others have identified important roles for both virus infections and early atopic sensitisation to inhalants in the development of the asthma phenotype during early childhood. Our recent findings that maximal risk is encountered only if sensitisation is present contemporaneously with these early viral infections, as opposed to simply having positive atopic family history. This argues that IgE itself is part of this risk profile, and the latter cannot be explained alone by indirect effects of the atopic phenotype such as Th1/Th2 imbalance in host antiviral immunity, although this does appear to be a significant cofactor.

We have recently followed these interactions through into established asthma, notably their role in acute severe exacerbations in children up to age 16yrs. The epidemiological literature indicates that the vast majority of such children are atopic and invariably virally infected, but the significance of these comorbidities has hitherto not been well understood. Based on the established literature indicating the operation of a lung:bone marrow axis during inflammation we hypothesized that signals from the lung during exacerbations should alter the functional phenotype of cell populations being released into the blood in a systematic fashion, thus initiating programming of cellular defence functions required at the challenge site. To address this hypothesis we used a genomics-based approach involving profiling of gene expression signatures in paired PBMC subpopulations from individual children collected during exacerbation versus convalescence by microarray and cytometry.

The most prominent signatures identified were from Type 1 IFNs and IL-4/IL-13, particularly amongst myeloid cells. In particular monocyte/DC populations displayed a range of phenotypic changes that have strong likelihood to enhance local inflammation in the atopic lung. In particular, upregulation of FcER1 which markedly amplifies capacity for allergen uptake/presentation to Th2-effector cells via IgE-mediated allergen capture, and secondarily programming of the IL-13R-associated "alternatively activated" phenotype. Our findings suggest that respiratory viral infection in atopic children may trigger an atopy-dependent cascade which amplifies and sustains airway inflammation initiated by innate anti-viral immunity via harnessing underlying atopy-associated mechanisms. The operation of this cascade may account for the uniquely high susceptibility of atopics to severe of viral-induced asthma exacerbations.

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A selective H_4R antagonist prevents antigen-induced airway inflammation in guinea pig: A pivotal role of annexin-A1

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Although mechanism involved in the pathogenesis of asthma remain unclear, role for oxidative/nitrosative stress have been documented. Recent evidences suggest that histamine has a key role in allergic inflammation through the activation of histamine H_4 receptor, a novel G-protein coupled receptor [1]. Annexin-A1 (lipocortin-1, LC-1), a 37 kDA anti-inflammatory protein that inhibits the activity of cytosolic phospholipase A_2 ($cPLA_2$), plays a key role in the production of lipid inflammatory mediators such as prostaglandings (PGs) and leukotrienes (LTs) [2].

Here we report the effects of compund JNJ 7777120 (JNJ), a selective H_4 receptor antagonist, on antigen-induced airway inflammation and LC-1 levels in brochoalveoar lavage (BAL) fluid.

Ovalbumin-sensitized guinea pigs placed in a respiratory chamber were challenged with the antigen. JNJ, at the dose of 5, 7.5 and 10 mg/Kg b.wt. was given i.p. for 4 days before OA challenge. Respiratory parameters were recorded and quantified. BAL fluid was collected and the lung removed from guinea pigs 48 h after OA challenge. In the BAL fluid, the levels of LC-1, PgD_2, LTB_4 and TNFα were determined. Myeloperoxidase and caspase-3 activities, 8-hydroxy-2-deoxyguanosine and MnSOD were evaluated in lung samples.

OA-challenge decreased significantly LC-1 levels in BAL fluid, associated with respiratory abnormalities and a significantly increase of PgD_2, LTB_4, and TNF-α levels. Treatments with JNJ significantly and dose-dependently increase the levels of LC-1, reduced cough, dyspnea and severe bronchoconstriction and the levels of PgD_2, LTB_4, and TNF-α in BAL fluid. Moreover inverse correlations among LC-1, prostanoid and cytokine levels in BAL fluid were observed.

These results suggest that antigen-induced asthma-like reaction decreases the levels of LC-1 with a consequent increase in TNF-α and eicosanoid production. JNJ pre-treatment modulate allergic asthmatic response and airway inflammation throughout an up-regulation of LC-1.

This research was supported by COST Action BM0806: Recent advances in histamine receptor H_4R research.

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ORAL ABSTRACT SESSION 10: Experimental Allergy

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Conformational change in IgE upon receptor binding as a basis for inhibitor design

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The interaction between IgE and FcεRI, the high affinity receptor on mast cells, basophils and antigen-presenting cells is a proven target for therapeutic intervention in allergic disease, as demonstrated by the success of the anti-IgE omalizumab. A small molecule inhibitor would offer significant advantages, but protein-protein interactions are generally considered difficult targets for small molecule inhibition, and the large surface area and high affinity make this case appear particularly intractable to such an approach. However, we have now determined by X-ray crystallography the structure of the complex between the complete IgE-Fc, consisting of the dimer of the Cε2, Cε3 and Cε4 domains, and the two soluble extracellular domains of FcεRIα. The structure reveals the precise nature of the interaction and the essential conformational changes that occur in IgE to generate high-affinity binding. The Cε2 domains were found to play an indirect role only, but their contribution may be understood through their effect upon the thermodynamics of the interaction, which was found to be almost entirely entropically driven. These structural data provide the information required to pursue an allosteric, rather than a direct blocking approach to inhibition, by preventing the conformational changes and locking the IgE molecule in an inactive state.

Contrary to expectations, the conformational changes in IgE that occur upon receptor binding lead to an even more compact and bent structure than that of free IgE. We have also confirmed this result in solution using FRET and fluorescence lifetime measurements with labelled IgE-Fc. The six domains of IgE-Fc alter their relative orientations, opening some and closing other sites for the binding of small molecule allosteric inhibitors. These sites have been targeted by *in silico* screening, and small molecules with inhibitory activity as determined by ELISA and Biacore analysis have been identified. Blocking the conformational change in IgE has the potential to reduce the affinity 1000-fold, and we have shown that this is sufficient to cause a profound reduction in anaphylaxis in a hFcεRI transgenic mouse model.

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An itch-producing sensory nerve population in the mouse

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The primary afferent nerve pathways involved in itch are largely unknown. Itch induced by chloroquine (CQ) is a common side effect of this widely used anti-malarial drug and is also a well-documented pruritic agent in experimental animals. Mrgprs are 7TM G-protein receptors that are selectively expressed on sensory nociceptors. We deleted a chromosomal locus spanning twelve Mrgpr genes in mice. The Mrgpr -/- mice behaved normally and responded normally in various assays that assess pain. By contrast, they displayed significant deficits in itch-associated responses induced by CQ. CQ directly evokes calcium increases in a small subset (~4%) of dorsal root ganglion sensory neurons from wild-type mice whereas Mrgpr-deficient neurons completely lose CQ sensitivity. Combining the functional calcium assay with single-neuron RT-PCR we noted that the CQ-sensitive, presumed "itch-causing" neurons, selectively express the MrgprA3 receptor mRNA. CQ can specifically activated mouse MrgprA3 and human MrgprX1 in a heterologous system. Moreover, these genes can confer CQ sensitivity to neurons isolated from the Mrgpr -/- mice. Therefore, activation of MrgprA3 is likely the mechanism underlying CQ-induced itch in mice. The MrGprA3 expressing itch-causing neurons represent a small subset (~18%) of the histamine responsive population of DRG neurons. Histamine-

responsive DRG neurons can be segregated into three subgroups based on their diameters (i.e. 12-17 μM, 18-25 μM, and 26-32 μM). The CQ-sensitive neurons fall into the 18-25 μm diameter subgroup and account for about 40% of this subpopulation. All of the MrgprA3 expressing, CQ-sensing, neurons are capsaicin-sensitive (express TRPV1). Thus, CQ-sensitive neurons in mouse DRG define a small subset of histamine- and capsaicin-sensitive neurons. We hypothesize that these neurons may represent an important subset of primary afferent nerve population specifically linked to itching. Supported by NIH, USA.

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Periostin, an extracellular matrix protein, acts as a master switch for the onset of inflammation in atopic dermatitis

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Atopic dermatitis (AD) is a chronic, relapsing, highly pruritic, inflammatory skin disease whose inflammation is characterized by dysregulated reactions of immune and non-immune cells; the former includes accumulation of cells expressing dominantly Th2 cytokines and increase of activated antigen-presenting cells driving Th2 differentiation and the latter includes production of proinflammatory cytokines and chemokines in keratinocytes. However, it remains undetermined how amplification and persistence systems of inflammatory responses involving immune and non-immune cells are regulated in AD. Here we show that periostin, an extracellular matrix protein downstream of the IL-4/IL-13 signals, acts as a master switch for inflammation in AD. IL-4/IL-13 induced secretion of periostin in dermal fibroblasts and then periostin directly acts on keratinocytes through α_V integrin, inducing their proliferation and differentiation in three-dimensional organotypic co-culture system using keratinocytes and fibroblasts *in vitro*. Moreover, keratinocytes activated by periostin produced proinflammatory cytokines including thymic stromal lymphopoietin (TSLP) via NF-κB, in turn inducing Th2 responses. Either *Stat6*$^{-/-}$ or *Postn*$^{-/-}$ mice or administration of neutralizing anti-integrin α_V antibodies impaired appearance of AD-like phenotypes in model mice generated by destroying skin barrier with tape stripping followed by painting mite antigen onto ears. Furthermore, periostin was expressed in the dermis of AD patients in proportion to the severity. These results demonstrate that immune cells (dendritic and Th2 cells), stromal cells (fibroblasts), and parenchymal cells (keratinocytes) compose a pathogenic loop in AD inflammation involving IL-4/IL-13, periostin, α_V integrin, and inflammatory cytokines inducing Th2 responses including TSLP, and this loop is crucial for the onset of AD inflammation in model mice.

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PLC-β3 deficiency causes skin inflammation

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Atopic dermatitis is a chronic pruritic inflammatory skin disease. Gene loci on human chromosome 11q13 are linked to atopic dermatitis, asthma, allergic rhinitis, and atopy. The *PLCb3* gene encoding phospholipase C-β3 (PLC-β3) and several candidate genes are localized to this region. Here we show by genetic association study that the *PLCb3* gene is significantly associated with atopic dermatitis and serum IgE levels. Importantly, *PLC-β3*$^{-/-}$ mice spontaneously develop atopic dermatitis-like skin lesions, with concomitant serum IgE elevation. Among skin-infiltrating cells, mast cells, but not T cells, are shown to be indispensable for the development of skin lesions. Allergen-induced dermatitis is more severe in mast cell-sufficient than mast cell-deficient mice and is more severe in *PLC-β3*$^{-/-}$ than wild-type mice. Remarkable abundance of mast cells in *PLC-β3*$^{-/-}$ mice was due to the loss of an adaptor function of PLC-β3 to inhibit IL-3-mediated proliferation of mast cells via SHP-1-mediated suppression of Stat5 activity. Therefore, this study demonstrates a critical importance of mast cells in spontaneous and allergen-induced dermatitis. We propose that risk of atopic dermatitis is controlled by elements of the pathways composed of a group of cytokines IL-3/IL-5/GM-CSF/TSLP that activate STAT5, PLC-β3 and SHP-1.

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Cord blood hemopoietic progenitor cell toll-like receptor expression and function: A mechanism underlying allergic inflammation in early life?

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Rationale: Neonatal immune responses to environmental stimuli may determine the development of atopy in childhood. There is now a burgeoning body of evidence showing that activation of selective hemopoietic processes is not only associated with the onset and maintenance of allergic inflammation in atopic adults, but also with the *development* of the allergic diathesis in infants. Functional and phenotypic alterations of hemopoietic progenitors relevant to eosinophil/basophil (Eo/B) lineage commitment have been observed in neonates at risk for atopy and asthma. Since atopy in early life may be modulated by the ligation of Toll-like Receptors (TLR) on immunocompetent cells, we have recently studied the expression of TLR on cord blood (CB) progenitors. Specifically, we investigated alterations in progenitor expression and differentiation profiles after stimulation with TLR agonists.

Methods: Freshly isolated, CD34-enriched human CB cells were stimulated with 10μg/mL lipopolysaccharide or 5μM CpG oligodeoxynucleotides overnight. Flow cytometric analyses were used to evaluate surface and intracellular expression of TLR-2, TLR-4, and TLR-9, as well as the hemopoietic cytokine receptors (HCR), IL-5R, IL-3R and GM-CSFR; methylcellulose cultures were performed to assess CD34+ cell differentiation capacity into Eo/B colony forming units (CFU).

Results: After TLR agonist stimulation, CD34+ cell TLR-2, TLR-4 and TLR-9 percentage expression increased significantly ($p=0.005$), whereas HCR expression decreased ($p=0.01$); however, mean fluorescence intensity of all receptors was found to be increased. Stimulation with a combination of TLR agonists and hemopoietic cytokines induced increased IL-5- and IL-3-responsive Eo/B CFU ($p=0.02$), when compared to hemopoietic cytokine stimulation alone. Moreover, in analyses of birth cohort samples, high atopic risk infants had significantly lower CB progenitor cell TLR-2, TLR-4 and TLR-9 expression, and significantly more IL-3-, IL-5- and GM-CSF-responsive Eo/B CFU.

Conclusions: CB CD34+ progenitor cells significantly express TLR, and TLR ligation directly affects both TLR and HCR expression. Maternal allergy may predispose infants to allergic risk through regulation of progenitor TLR expression and function *in utero*. These findings may highlight an alternate innate immune pathway of microbial influence on the development of allergic inflammation in early life.

Abstracts

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Active immunization with mimotopes mimicking the extracellular membrane-proximal domain (EMPD) of mIgE leads to the generation of anti-mIgE antibodies *in vivo*

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Today, almost 30% of the population of Europe and the USA suffer from undesirable allergic reactions. Since the mid-90's, anti-IgE therapy has become a powerful method of treatment. Anti-IgE antibodies prevent IgE from binding to its high affinity receptor FcεRI on mast cells and basophils, therefore avoiding the release of mediators that cause allergic symptoms. Nevertheless, these anti-IgE antibodies have the potential to interfere with the whole repertoire of IgE antibodies that has been generated during previous infections and which may have biological relevance. To keep the immunological competence untouched, we try to go one step further by addressing B cells expressing membrane-bound IgE receptors.

Previously, we could show that passive immunization of mice with our monoclonal antibody mAbA9, specific for the EMPD of mIgE, during antigen sensitization led to a dramatic decrease of specific IgE titres. Screening of a mimotope phage library, displaying random decamer peptides, with mAbA9 resulted in the isolation of peptides that successfully mimicked the EMPD region. By means of mimicking certain epitopes on allergens, mimotopes should be able to induce the generation of epitope-specific antibodies. Sequences of positive screened mimotopes were used for synthesis and affinities of these peptides were measured with BIACORE X device. Finally, mimotopes were used for active immunisation experiments.

Mice of experimental groups were immunised for four times every two weeks, whereas mice of the control group only received PBS. During the course of immunization an increase of IgG1 titres specific for the mimotopes could be observed. Moreover, mice sera were also positive for the corresponding EMPD peptide itself, indicating that mimotope immunization indeed generated anti-mIgE-specific antibodies *in vivo*.

To investigate whether these previous, mimotope-induced anti-mIgE secreting plasma cells can clear newly synthesized, allergen-induced IgE B cells, subsequent immunization with sensitizing antigen Bet v 1a was performed. First results, which show a decrease of Bet v 1a-specific IgE titres in mimotope-treated mice, indicate that mimotope-induced anti-mIgE antibodies have the potential to successfully interfere IgE production.

POSTER SESSION 3: Experimental Models of Inflammatory and Allergic Diseases

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Investigating the role of osteopontin in psoriasis: Immunoregulation of antimicrobial peptides

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Background: Osteopontin (OPN) is a phosphorylated acidic glycoprotein produced by cells of the immune system, epithelial tissues, smooth muscle cells, osteoblasts, and tumor cells. OPN is chemotactic, supporting the adhesion and modulating the function of T cells and monocytes/macrophages.

Recent research has defined a role for OPN as a regulator of inflammatory cell accumulation and function at sites of inflammation and repair, influencing the polarization of Th cells. OPN interacts with integrins and CD44 to enhance Th1 and inhibit Th2 cytokine expression.

In psoriasis, it has recently been demonstrated the involvement of Th17 cells. These cells are characterized by the production of proinflammatory cytokines IL-17 e IL-22. A critical role is also played by IL-23, due to its ability to drive Th17 cell expansion.

IL-22 and IL-17 have been shown to induce keratinocyte gene expression of antimicrobial peptides or proteins (AMPs): β-defensins, LL-37.

AMPs represent an ancient and efficient innate defense mechanism which protects interfaces from infection with pathogenic microorganisms. In human skin AMPs are produced mainly by keratinocytes, neutrophils, sebocytes or sweat glands and are either expressed constitutively or after an inflammatory stimulus.

Objective: To investigate the relationship between OPN and AMPs in human psoriasis.

Materials and methods: Paired skin biopsies from lesional and non-lesional skin of psoriatic patients were used for quantitative polymerase chain reaction (qPCR) and immunohistochemistry. Peripheral blood mononuclear cells (PBMC) from psoriatic patients and healthy donors were analyzed by qPCR. Activation assays were performed with primary human keratinocytes.

Results: In our study population OPN is overexpressed in psoriatic PBMC and skin biopsies respect to controls. A quite high expression of OPN was also detected in non-lesional skin of psoriatic patients. OPN staining was both expressed in keratinocytes and in dermal infiltrating lymphocytes. β-defensin 2 and LL-37 were highly expressed in lesional psoriatic skin.

Conclusions: A new immunoregulatory role of OPN in psoriasis is showed, implying the possibility of therapeutical perspectives.

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Expanding the view of allergenicity: Adjuvant, non-allergenic factors from pollen regulate disparate signaling pathways in human target cells resulting in a proallergenic micromilieu

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We recently identified mediators from pollen acting on human dendritic cells and keratinocytes in a way that leads to the development of a Th2-dominated proallergenic micro milieu. In this study we were able to expand these findings by depicting signal transduction pathways in human target cells. Aqueous pollen extracts (APE) as well as pollen associated phytoprostanes (PPE_1)

inhibit IκBα degradation and p65 nuclear translocation in a PPAR-γ dependent manner in dendritic cells. Another Th2 promoting effect of APE on DCs was revealed in the down-regulation of Notch-Ligands Delta-1 and –4 while inducing Jagged-2.

To exclude protein components, low molecular weight APE-fraction was generated. Mass spectrometry revealed over 300 substances in APE<3kDa. This fraction activates DCs to release cAMP. By HPLC we detected here purine nucleosides, foremost adenosine. Since adenosine is a known modulator of DC function, DCs were stimulated with APE or adenosine in the absence or presence of adenosine receptor antagonists. Both adenosine and APE lead to inhibition of IL12p70. This inhibition was partly blocked by an A2B antagonist or by digestion of APE with adenosine deaminase. These results were mirrored in allogenic MLR, where APE-stimulated dendritic cells exhibited a reduced capacity to differentiate Th1 cells, an effect which was clearly attenuated after enzymatic digestion of pollen-associated adenosine. Thus, alongside the hitherto identified pollen associated PPE_1, we revealed other pollen-associated substances <3kDa such as adenosine that most likely add to the strong Th2 polarizing effect of pollen.

In order to further investigate relevant target cells of pollen derived mediators keratinocytes were stimulated by pollen extracts (<3kDa). Here we revealed that low-molecular mediators significantly induced the release of GM-CSF and IL1beta in a MAP-Kinase (p38) dependent manner. These results implicate that pollen not only act both on dendritic cells and human keratinocytes but also impact importantly the keratinocyte – dendritic cell axis by inducing dendritic cell-stimulating factors in keratinocytes.

Collectively, pollen grains represent packages of danger signals that influence the outcome of immune responses in many ways. For a better understanding of allergenicity, we integrated in this study the role of non-allergenic cofactors present during allergen exposure that favour the development of allergic sensitization and disease.

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Eoxins in asthma in children - A new pathway of inflammation

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Background: Increased levels of leukotrienes in exhaled breath condensate (EBC) have been reported in asthma and bronchial hyper-responsiveness (BHR), but little focus has been put on eicosanoids generated through the 15-lipoxygenase (LO) pathway (15-HETE and eoxins).

Objective: To investigate the relationship between metabolites of the 5- and 15-LO pathway in EBC and childhood asthma, asthma severity and clinical asthma parameters.

Methods: The present cross sectional study included 131 school children; 27 defined as problematic severe asthma, 80 mild-to-moderate asthma and 24 non-asthmatic controls. Clinical examination included spirometry, fractional exhaled nitric oxide (FE_{NO}), skin prick tests and methacholine bronchial challenge. Eicosanoids from the 5- and 15-LO pathways were analysed in EBC using tandem mass spectrometry (MS) and gas chromatogarphy. EBC eicosanoid/palmitic acid (PA) ratios were used in data analysis to adjust for sample dilution.

Results: Eoxin C_4-, D_4-, E_4-, 15-HETE- and LTC_4/PA-ratios were significantly increased in children with vs. without asthma; eoxin D_4- and LTE_4/PA-ratios were significantly higher in children with BHR to methacholine. A non-significant trend was observed towards higher eoxin/PA-ratios with increasing asthma severity. Eoxin E_4/PA-ratio was significantly higher in children with allergic sensitisation.

Conclusion: The results point to increased activity of the 15-LO inflammatory pathway in childhood asthma. MS analyses of EBC demonstrate that elevated eoxins not only accompany the increased 5-LO product LTC_4, but are also associated with BHR. The present study is the first to demonstrate an association between eoxins and asthma and bronchial hyper-responsiveness in children, potentially signifying a new therapeutic target for asthma treatment.

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***Helicobacter pylori* Hp(2-20) promotes gastric mucosal healing and interacts with formyl-peptide receptors**

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***Helicobacter pylori*-derived peptide RpL1 aa 2–20 [Hp(2–20)] in addition to its antimicrobial action exerts several immunomodulatory effects in eukaryotic cells by interacting with formyl peptide receptors (FPRs). It has recently been shown that activation of FPRs facilitates intestinal epithelial cell restitution. We investigated whether Hp(2-20) induces healing of injured gastric mucosa and assessed the mechanisms underlying any such effect. We investigated the expression of FPRs in two gastric epithelial cell lines (MKN-28 and AGS) at mRNA and protein level. To determine whether FPRs were functional we performed chemotaxis experiments and proliferation assays and studied the Hp(2-20)-activated downstream signaling pathway. The effect of Hp(2-20) on mucosal healing was evaluated in rats after indomethacin-induced injury. Here we show that: (1) FPRs were expressed in both cell lines; (2) Hp(2-20) stimulated migration and proliferation of gastric epithelial cells; (3) this effect was specifically mediated by formyl peptide receptor-like 1 (FPRL1) and FPRL2 and was associated with activation of FPR-related downstream signalling pathways; (4) Hp(2-20) up-regulated the expression and secretion of vascular endothelial growth factor; and (5) Hp(2-20) accelerated healing of rat gastric mucosa after injury brought about by indomethacin at both the macroscopic and microscopic levels. In conclusion, by interacting with FRPL1 and FPRL2, *H. pylori*-derived Hp(2-20) induces cell migration and proliferation, as well as the expression of vascular endothelial growth factor, thereby promoting gastric mucosal healing. This study provides further evidence of the complexity of the relationship between *H. pylori* and human gastric mucosa, and it suggests that a bacterial product may be used to heal gastric mucosal injury.**

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Ovalbumin nitration is associated with a reduced risk for de-novo sensitization via the oral route in a murine food allergy model

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Background: Recently, nitration e.g. by ambient pollutants was revealed to increase the allergic potential of pollen allergens. Additional to environmental pollution also nitration processes during inflammatory responses could directly influence protein immunogenicity and might therefore contribute to food allergy induction. Thus, in the current study we aimed to analyze the impact of protein nitration on food allergy induction via the oral route.

Methods & results: BALB/c mice were immunized intragastrically by feeding nitrated and sham-nitrated ovalbumin (OVA) with or without concomitant acid-suppression. To analyze the impact of the sensitization route, mice were injected the allergens intraperitoneally. Animals being fed sham-nitrated OVA under acid-suppressive medication developed significantly elevated levels of IgE, and increased titers of specific IgG1 and IgG2a antibodies. Interestingly, oral immunizations of nitrated OVA under anti-acid treatment did not result in IgE formation. In contrast mice being immunized intraperitoneally revealed high levels of OVA specific IgE, which were significantly increased in the group being injected nitrated OVA. Interestingly, digestion experiments simulating physiological gastric conditions demonstrated protein nitration to interfere with protein stability as nitrated OVA was easily degraded, whereas sham-nitrated OVA remained stable up to 120 min.

Conclusions: These data indicated that nitration of OVA may be associated with a reduced allergenic capacity via the oral route due to enhanced protein digestibility.

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Mast cell tryptase as a mediator of inflammation through mechanisms independent of protease activated receptor 2

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BACKGROUND: The serine protease tryptase is secreted in substantial quantities from human mast cells. This protease can induce profound alterations in the behaviour of several cell types which are consistent with roles for tryptase as a mediator of inflammation and tissue remodelling. Protease activated receptor 2 (PAR-2) is the only cell receptor for tryptase to be described to date, but its precise contributions in tryptase-induced inflammation remain unclear.

METHODS: Recombinant human β-tryptase was injected into the peritoneum of PAR-2 knockout and wild type control C57BL/6 mice. At 6, 12 and 24 hours following injection, mice were killed and peritoneal lavage performed. Nucleated cells were enumerated and differential cell counts performed. Concentrations of albumin and protein were determined in peritoneal lavage fluid, and gelatin zymography was carried out. In separate experiments, neutrophils in peritoneal lavage fluid were isolated (using an immuno-magnetic procedure) and incubated with tryptase prior to analysis of supernatants by gelatin zymography.

RESULTS: Tryptase induced significant peritoneal neutrophilia, and was associated with elevated concentrations of albumin and protein in lavage fluid consistent with increased microvascular leakage. No significant differences between PAR-2 knockout and wild-type mice were observed in the cellular responses or in the microvascular leakage elicited by tryptase. Pre-incubation of the enzyme with selective inhibitors of tryptase, or heat inactivation of the enzyme, resulted in less neutrophil accumulation and lower albumin concentrations. A peptide agonist of PAR-2 (H-Ser-Leu-Ile-Gly-Arg-Leu-NH_2) failed to replicate the actions of tryptase, and injection of peptides corresponding to the PAR-2 exodomain was without effect. Increased concentrations of matrix metalloproteinases (MMP) 9 and 2 were detected by zymography in peritoneal lavage fluid of tryptase-injected mice of both strains. The MMP9 levels detected were correlated closely with neutrophil numbers in the peritoneum. Addition of tryptase to purified peritoneal neutrophils resulted in increased secretion of MMP9 suggesting that neutrophils were the primary source of this protease.

CONCLUSION: Tryptase is a potent stimulus for neutrophil recruitment, MMP release and microvascular leakage *in vivo*. Though dependant on the catalytic activity of tryptase, the mechanism involves processes independent of PAR-2 activation.

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Heterogeneity of the effects of radiocontrast media on mediator release from human mast cells and basophils

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More than 10 million radiologic examinations using intravascular radiocontrast media (RCM) are performed per year in the United States. Up to 1% of all individuals receiving RCM experience anaphylactoid reactions involving some combination of pulmonary, cardiovascular and cutaneous symptoms. The activation of basophils and mast cells (MC) plays a role in the pathogenesis of anaphylactoid reactions occurring during the administration of RCM. We compared the effects of three RCM, Hexabrix (sodium and meglumine salts of ioxaglic acid), Telebrix (sodium and meglumine salts of ioxitalamic acid), and Optiray (ioversol) on the release of preformed (histamine and tryptase) and *de novo* synthesized (PGD_2 and LTC_4) mediators from basophils and MC purified from human lung (HLMC), skin (HSMC), and heart tissue (HHMC). Hexabrix (0.1 to 0.3 mol/L), Telebrix (0.1 to 0.5 mol/L), Optiray (0.2 to 0.5 mol/L) concentration-dependently induced HR from basophils. A positive correlation was found between RCM osmolality and HR from basophils. Mast cells isolated from different anatomic sites responded differently to the three RCM. Hexabrix and Optiray induced histamine and tryptase release from HLMC, but not from HSMC. No correlation was found between the osmolality of RCM and HR from HLMC. There was a significant correlation between the percent of histamine and tryptase release induced by RCM from HLMC. Hexabrix, Telebrix, and Optiray also induced histamine and tryptase release from HHMC. None of the RCM induced the *de novo* synthesis of LTC_4 or PGD_2 from basophils or MC. RCM-induced HR from basophils and HLMC was temperature-dependent and partially influenced by extracellular Ca^{2+} concentrations. These results provide evidence of the

heterogeneity of the effects of RCM on mediator release from human basophils and MC from different anatomic sites. The variability of the response of basophils and MC from different donors to the same RCM was due to the releasability parameter. Hyperosmolality is not the only factor in the activation of basophils and MC by RCM. RCM induce only the release of preformed mediators. Given the longer half-life of tryptase than histamine in plasma, measurements of plasma tryptase may become a useful diagnostic tool for identifying adverse reactions to RCM.

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Structural damage of surfactant protein-D (SP-D) in ozone (O3)-induced exacerbation of allergic airway inflammation is associated with dcndritic cell activation

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RATIONALE: Ozone (O_3) promotes exacerbation of the asthmatic airway inflammation, likely due to failure of protective immune mechanisms. SP-D is one of the critical pulmonary innate immune regulators with an incompletely understood dual function on innate immune cells: A suppressive effect is attributed to SP-D binding to signal regulatory protein (SIRP)-α, an inhibitory membrane receptor while activation of the calreticulin/CD91 complex induces proinflammatory signals. We hypothesized that following O_3 inhalation the highly oligomerized native SP-D molecule undergoes oxidative damage and conformational changes that can affect its immunoprotective function.

METHODS: To study the effects of O_3 exposure on allergic airway inflammation Balb/c mice were sensitized and challenged with *Aspergillus fumigatus* (*Af*). Four days later the mice were also exposed to O_3 (3.0 ppm for 2 h). The bronchoalveolar lavage (BAL) supernatant was assessed for cytokine/chemokine profile and release of calreticulin. SP-D conformational changes were studied by native gel electrophoresis using an in-house biotinylated monoclonal anti-SP-D. BAL cell SP-D binding, cell activation and the effects of SP-D on bone-marrow derived dendritic cells were investigated by immunocytochemistry and FACS analysis in wild type and SP-D-/- mice.

RESULTS: O_3 exposure induced the appearance of abnormal, trimeric SP-D in the BAL fluid. Exacerbation of Th2-type airway inflammation including enhanced eosinophilia, IL-5, GM-CSF, TNF-α and IL-6 release occurred in previously allergen treated but not in naïve mice. Activation of CD11c/CD11b+ dendritic cells was characterized by increased expression of the proinflammatory calreticulin/CD91 complex. Lack of SP-D in gene targeted mice by itself caused migration and activation of myeloid dendritic cells. These animals also showed heightened susceptibility to and prolonged resolution of allergen and O_3- induced inflammation. In contrast, treatment of bone marrow-derived dendritic precursors with recombinant SP-D significantly inhibited maturation and autocrine TNF-α and Ccl17 release. SP-D-/- BAL cells were highly heterogenous with only a small subset expressing SIRP-α. SIRP-α+ cells bound exogenous recombinant SP-D in a dose-dependent manner.

CONCLUSIONS: O_3-induced conformational impairment of SP-D can predispose to proinflammatory alterations of airway dendritic cells. A loss of ability to bind SIRP-α maybe responsible for diminished SP-D immunoprotection during exacerbation of the allergic airway changes after O_3-exposure.

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The activin A-binding protein follistatin inhibits airway remodelling in a murine model of chronic asthma

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This study aimed to investigate the therapeutic potential of follistatin, the soluble binding protein of activin A, to treat or prevent airway remodelling and inflammation in a chronic murine model of experimental asthma.

Background: Asthmatic airway remodelling is thought to contribute to asthma severity and declining lung function. The immunoregulatory cytokine TGF-beta is a major driver of the asthmatic airway remodelling process. Activin A, a member of the TGF-beta superfamily, has recently been shown to have immunoregulatory and pro-fibrotic properties.

Methods: We tested whether follistatin, an endogenous inhibitor of activin A bioactivity, could inhibit allergic inflammation and remodelling in a murine chronic asthma model. Mice were systemically sensitized with ovalbumin (OVA) in alum, and challenged intranasally with OVA or OVA mixed with follistatin (0.05, 0.5 or 5 mcg) three times/week. Analysis was performed after 1, 3 and 5 weeks of allergen challenge.

Results: Compared to the OVA group, mice treated with OVA+0.5 mcg follistatin had significantly decreased total lung leukocyte counts at 5 wks. Serum OVA-specific IgE concentrations were significantly decreased in the OVA+5 mcg follistatin group at 3 weeks. Mice treated with OVA+5 mcg follistatin had significantly decreased bronchoalveolar lavage activin A and TGF-beta concentrations at 5 wks, coinciding with significantly decreased subepithelial collagen deposition. These data are the first to show that the activin A antagonist follistatin can attenuate asthmatic airway remodelling.

Conclusion: Our findings suggest that follistatin could be used as a therapeutic to treat or prevent airway remodelling in asthma and other inflammatory and/or fibrotic lung diseases where activin A is implicated in pathogenesis.

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A pathogenic mechanism of eczema vaccinatum

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Threats of bioterrorism have renewed efforts to better understand poxvirus pathogenesis and to develop a safer vaccine against smallpox. Individuals with atopic dermatitis are

excluded from smallpox vaccination due to their propensity to develop eczema vaccinatum, a disseminated vaccinia virus (VACV) infection. To study the underlying mechanism of the vulnerability of atopic dermatitis patients to VACV infection, we developed a mouse model of eczema vaccinatum. Virus infection of eczematous skin induced severe primary erosive skin lesions, but not in skin of healthy mice. Eczematous mice exhibited lower natural killer (NK) cell activity, but similar cytotoxic T lymphocyte activity and humoral immune responses. The role of NK cells in controlling VACV-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 atopic dermatitis patients, these results can explain the increased susceptibility of atopic dermatitis patients to eczema vaccinatum.

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Alveolar macrophages inhibit both Th1 and Th2 immune response and induce airway remodeling in intranasally sensitized mouse asthma model

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Background: Resident alveolar macrophages (AM) are predominant immune cells in the alveolar spaces and airways. In Th2-mediated mouse model, AM activated by IL-4 or IL-13 can express resistin-like molecule-alpha (RELM-alpha). It has been discovered that RELM-alpha inhibit Th2-cytokine production from Th2 cells and drive proliferation and growth factor expression in lung fibroblast cell lines. However, the relationship between AM and RELM-alpha in inhibiting allergic inflammation and in inducing airway remodeling is not fully elucidated.

Objective: To investigate whether resident AM could regulate Th2 inflammation and airway remodeling via RELM-alpha-mediated pathway.

Methods: C57BL/6 mice were intranasally sensitized by OVA with O.1ug LPS four times (day 1, 2, 3, and 7) (LPS/OVA group) and then challenged with LPS-depleted OVA on day 14 and 15. Control mice were treated with PBS only (PBS group). Twenty-four hour after last challenge, bronchoalveolar lavage (BAL) and excision of lung for RNA extraction were performed. The change in the expression of Th1/Th2 cytokines (IL-4, IL-5, IL-12p70 and IFN-gamma) and RELM-alpha were measured with Bio-plex assay and ELISA. The expression of downstream molecules (alpha-Smooth muscle actin (alpha-SMA), Surfactant Protein-B and –C (SP-B and –C)) induced by RELM-alpha were analyzed with real-time PCR. To confirm the results, we repeated the same procedures after depleting AM by 3-day injection of liposome-encapsulated clodronate via intranasal route before challenge.

Results: In AM-depleted LPS/OVA group, the number of total inflammatory cells did not change, but the number of neutrophils and concentrations of Th1 and Th2 cytokines (IL-4, IL-12p70 and IFN-gamma) significantly increased in BAL compared with Sham after challenge with OVA. In addition the protein and mRNA expression of RELM-alpha notably decreased in AM-depleted LPS/OVA group compared with Sham, accompanied by a similar reduction in the expression level of alpha-SMA and SP-C.

Conclusion: This study demonstrates that alveolar macrophage can suppress both Th1 and Th2 immune response and induce the expression of airway remodeling factors such as alpha-SMA in a mouse asthma model via RELM-alpha-mediated pathway, suggesting that AM participate in airway repair process which resolve inflammation and restore barrier integrity.

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Mechanistic basis for IgE-mediated Facilitated Antigen Presentation (FAP) and inhibition by allergen-specific IgG

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Background – Activation of allergen-specific T-cells occurs at very low allergen concentrations due to IgE-mediated Facilitated Antigen Presentation (FAP). Through FAP IgE may be a key regulator for T-cell activation and for development and perpetuation of the allergic immune response.

FAP inhibition by immunotherapy-induced allergen-specific non-IgE antibodies has been shown to correlate with clinical responses to immunotherapy. Here we demonstrate that formation of CD23/IgE/Derp2 complexes (FAP complexes) and their sensitivity to inhibition by non-IgE antibodies depend on allergen-specific IgE repertoire complexity, predominantly IgE clonality and affinity.

Methods – Patient sera or different combinations of recombinant Derp2-specific IgE antibodies with defined affinities were used for FAP complex formation on CD23+ B-cells *in-vitro*. A panel of recombinant anti-Derp2 IgG antibodies was used to assess the inhibitory effect of allergen-specific IgG on FAP complex formation. FAP complex formation was measured by FACS or by FAP-mediated T-cell activation using CD23+B-cells as APC for stimulation of Derp2-specific T-cell lines.

Results – FAP complex formation and subsequent T-cell activation occur in the presence of only two IgE antibodies of even low- to intermediate affinities that bind non-overlapping epitopes on Derp2, but are strongly favored by increased affinitiy of individual IgE's and, most prominently, by increased IgE-clonality. T-cell activation correlates consistently with FAP complex formation.

FAP complexes formed with low-complexity IgE-repertoires are less robust and more easily inhibited by allergen-specific IgG than complexes formed with IgE repertoires of higher complexity. FAP complexes formed by high-titered patients' sera are inhibited by allergen-specific IgGs in a manner reminiscent of high IgE-repertoire complexity whereas FAP complex formation by low-titered patients' sera is reminiscent of low IgE-repertoire complexity.

Conclusions – FAP complex-formation and inhibition is strongly affected by IgE repertoire complexity. Inhibition of FAP complexes by allergen-specific IgG leads to subsequent reduction of allergen-specific T-cell activation which may prevent or blunt the progression of allergic inflammation. Thus, IgE-titers seem to correlate with IgE-repertoire complexity and FAP complex robustness suggesting that early intervention by immunotherapy may be the most favorable treatment option. We have now demonstrated that IgE repertoire complexity most likely affects both the immediate allergic reaction (through effector-cell activation) and the T-cell mediated late-phase reaction (through FAP).

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The nitrosative stress in chronic nonproductive cough patients with increased nociceptive sensitivity

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Background: Nociceptor hyperreactivity of the upper airways has been suggested to induce chronic nonproductive cough. Reactive oxygen species are supposed to stimulate capsaicin-sensitive nerves innervating the lungs. Recently, an increase in ntinociceptive tolerance was reported to be associated with nitrosative stress in the murine model of spinal cord disease.

Objective: We hypothesized that nitrosative stress may enhance nociceptive sensitivity of the upper airways in chronic cough patients.

Methods: Twenty six patients with nonproductive cough as a sole clinical manifestation and 14 control subjects were enrolled. Capsaicin cough provocation test was performed to determine nociceptive sensitivity. To assess nitrosative stress in the upper airways, nasal nitric oxide (nNO) was measured and 3-nitrotyrosine (3-NT) in nasal epithelial cells was immunostained. The relationship between nociceptor sensitivity and the degree of nitrosative stress was analyzed. To investigate the effect of nitrosative stress on the airway epithelium, primary human nasal epithelial cells (HNECs) and BEAS-2B human bronchial epithelial cells (BECs) were employed to measure the level of substance P (SP) after treatment of NO donor (PAPA-NONOate) and hydrogen peroxide (H_2O_2) with/ without their inhibitors.

Results: The patients could be divided into 2 groups according to the results of the

capsaicin provocation test: an increased nociceptive sensitivity group (INS,

C5<32umol/L) and a normal nociceptive sensitivity (NNS) group. The levels of

Immunoreactivity for 3-NT in nasal brush cells and the values of nNO were

significantly higher in subjects with INS than those with NNS and control subjects.

The expression of SP was greatly enhanced in HNECs and BECs exposed to PAPA-

NONOate and H_2O_2.

Conclusions: Nitrosative stress in the upper airways may play a role in the pathogenesis of chronic nonproductive cough by enhancing nociceptor sensitivity. Therapeutic strategy reducing nitrosative stress may be beneficial to some chronic cough patients.

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Analysis of T cell-dependent bronchoconstriction using human cultured bronchial smooth muscle cells

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Background: Reversible airflow limitation is characterictic of bronchial asthma. To delineate IgE-independent mechanisms of asthma, T cell-dependent bronchoconstriction was investigated using an *in vitro* contraction assay of cultured human bronchial smooth muscle cells (hBSMC) and an adoptive transfer of murine Th clones *in vivo*.

Methods: Peripheral blood mononuclear cells (PBMC) obtained from nonatopic asthmatics were cultured with secreted aspartic proteinase 2 (SAP2), a purified protein derived from *Candida albicans*. SAP2-reactive Th clones were established by antigen stimulation followed by the limiting dilution. Cytokine concentration of the culture supernatants was measured by specific ELISAs. The contractile responses induced by the supernatants were analyzed using collagen gels embedded with cultured hBSMC. A panel of murine ovalbumin (OVA) specific Th clones were established from the regional lymphnodes of Balb/c mice immunized with OVA/ CFA and splenocytes of DO11.10 transgenic mice expressing T cell receptor specific for OVA/H-2^d. Th clones were adoptively transferred into unprimed mice. After antigen challenge, airway resistance was analyzed by resistance/compliance analyser under anesthetized condition.

Results: PBMC obtained from several nonatopic asthmatics produced significant amount of IL-5, IL-13, and IFN-γ upon incubation with SAP2 (responders). Upon bronchial challenge with SAP2, late but not immediate bronchial response was induced for the responders. Neither IAR nor LAR was detectable for the control asthmatics (nonresponders), indicating the specificity of the responses. The supernatants of PBMC cultured with SAP2 and Th clones atimulated with immobilized anti-CD3 Ab induced a significant and reproducible contraction of the gels embedded with cultured hBSMC. Mice transferred with an OVA-specific Th clone, T6-2, and challenged with OVA showed airflow limitation in terms of BUXCO unrestrained body plethysmography and resistance/compliance analyser under the restrained, anesthetized, and mechanically ventilated condition. The airflow limitation was induced not only by OVA but also by OVA peptide 323-339, the T cell epitope.

Conclusion: Our results clearly demonstrated that activation of Th cells is associated with airflow limitation. Nonatopic asthma may be caused by an IgE-independent, T cell-dependent immune-recognition. Identification of T cell derived bronchoconstrictor(s) seems promising especially for currently therapy resistant asthma.

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The participation of serine protease in the development of allergic airway inflammation in mice

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In order to clarify the participation of serine proteases in the development of allergic airway inflammation, we investigated the effect of nafamostat mesilate, a novel serine protease inhibitor, in a murine model of allergic asthma. Mice were sensitized by the intraperitoneal injection of ovalbumin (OA) with alum and then exposed to 1% OA for 30 min, three times every 4th day. Nafamostat mesilate was administered orally for 10 days during the allergen challenge. In sensitized mice, repeated allergen challenge induced an increase in tryptase proteolytic activity in blonchoalveolar lavage fluid (BALF). In addition, marked increases in inflammatory cells, level of Th2 cytokines and eotaxin in BALF, number of goblet cells in the epithelium, and level of OA-specific IgE in serum were observed after repetitive allergen inhalation. Treatment with nafamostat mesilate significantly inhibited not only increased proteolytic activities but also increases in the number of eosinophils and lymphocytes in the BALF. Nafamostst mesilate also inhibited

increses in the level of interleukin 13 and eotaxin in BALF and goblet cell hyperplasia in a dose related manner. These findings suggest that increased serine protease activity in the airways is involved in the development of antigen-induced allergic eosinophilic inflammation and epithelial remodeling in bronchial asthma.

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More than 99 percent of allergen-specific serum-IgE in the allergic patient does not originate from circulating plasma cells

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Background: Allergen-induced type I reactions are highly dependent on allergen-specific IgE antibodies, which are present perennially in allergic patients both as free serum IgE and bound to effector cells. In humans, the major site of continuous IgE production has not been identified so far.

Objective: In this study we aimed to investigate to what extent B cells or terminally differentiated plasma cells in the peripheral blood of allergic patients contribute to allergen-specific serum IgE levels.

Methods: PBMCs were isolated from patients allergic to birch pollen, grass pollen or house dust mite. Magnetically labelled antibodies directed against various cell surface markers were used to deplete or isolate specific cell populations. Allergen-specific IgE was measured in serum samples and 7-day cell culture supernatants by ELISA or by the ImmunoCAP system. RT-PCR reactions were performed to measure IgE synthesis directly on the mRNA level.

Results: We found that allergen-specific IgE present in the peripheral blood accounts for 99.9% of free, unbound serum-IgE, whereas only 0.02 % represents newly synthesized IgE. IgE antibodies found in PBMC cell culture supernatants mainly originate from basophils and account for 0.05 % when compared to specific serum-IgE levels.

Conclusion: We conclude that basophils are constantly and fully loaded with IgE bound to FcεRI on their surface and that the major production site of IgE does not reside in the peripheral blood.

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SERCA-2 expression in airway smooth muscle is altered by inflammatory cytokines and formoterol

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Airway hyper-responsiveness and remodelling are hallmarks of asthma, and airway smooth muscle (ASM) may be central to the pathogenesis of these abnormalities. Many functions of ASM, including contraction, cell migration and secretion of cytokines, are dependent on calcium signalling. Indeed, it has been proposed that altered function of ASM in asthma has been linked to dysregulation of intracellular calcium homeostasis. The sarcoendoplasmic reticulum calcium ATPase (SERCA) replenishes the sarcoplasmic reticulum calcium store, and is thus critical for regulating cytosolic calcium levels. We have recently reported that ASM derived from subjects with asthma exhibit a reduction in SERCA-2 expression, which translates to changes in ASM calcium handling and function *in vitro* (Mahn *et al*, *Proc Natl Acad Sci* 2009;106:10775–10780). In this study we investigated factors that may affect SERCA-2 expression, specifically the asthma-associated mediators IL-13 and TGFβ, and short and long acting beta2-agonists (salbutamol and formoterol). ASM was derived from endobronchial biopsies of healthy volunteers, as approved by the local ethical committee and with informed consent.

Methods: ASM cells were cultured from 8 subjects and experiments performed following 72hr growth arrest. Cells were stimulated for 24hr with IL-13 (10ng/ml), TGFβ (10ng/ml) or in combination, salbutamol (1-30μM) or formoterol (1-30nM). Expression of SERCA-2 was then examined using Western blot.

Results: Healthy ASM treated with IL-13, TGFβ and the combination showed reduced SERCA-2 expression by 21%±3, 47%±3 and 24%±6 respectively (c.f. to unstimulated, $p<0.05$). Salbutamol had minimal effects on SERCA-2 expression. ASM cells treated with formoterol at 1nM, 10nM and 30nM reduced SERCA-2 expression levels by 43%±9, 55%±1 and 63%±5 respectively (c.f. to unstimulated, $p<0.05$). Chronic treatment with formoterol has previously been reported to reduce SERCA-2 expression in the heart (Ryall *et al*, *Am J Physiol* 2008;294:H2587-H2595).

Conclusion: Inflammatory cytokines may underlie the reduced expression of SERCA-2 in asthmatic ASM, but interestingly treatment with long-acting beta2 agonists has a similar effect and this may have implications for therapy.

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Role of lung apolipoprotein A1 in development of asthma: Anti-inflammatory and anti - fibrotic effect

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Rationale: Asthma is caused by alterations in expression of proteins involved in multiple pathways, including allergic inflammation, injury, and repair.

Objectives: To understand the pathogenic changes in lung protein expression in asthma and to evaluate apolipoprotein A1 as a candidate therapeutic molecule.

Methods: Two-dimensional electrophoresis was adopted for differential display proteomics. RT-PCR, western blotting, immunohistochemical staining, and ELISA were performed for identification and quantitative measurement of apolipoprotein A1 and IL-5, IL-13, INF-gamma in lavage fluids from subjects with asthma and OVA-sensitized/challenged mice.

Main Results: Twenty protein spots showed differences in relative intensity between asthma and normal controls. MALDI-TOF/TOF mass spectrometry revealed increased Gc protein and decreased of apolipoprotein A1, α1-antitrypsin, macrophage capping protein, Glutathione S-transferase in asthma compared to normal controls ($P<0.05$). Apo A1 concentrations were lower in lavage fluids from subjects with asthma ($n=55$) than in normal controls ($n=18$, $P=0.009$). In OVA-sensitized/challenegd mice, Apo A1 protein and m-RNA in lung lysates was lower than that in sham-treated controls. Intranasal treatment with Apo A1 protein reduced the OVA-induced increases in number of inflammatory cells and goblet cells and Th2

cytokines including IL-5 and IL-13 in experimental asthma model in a dose-dependent manner ($P<0.05$).

Conclusions: Alterations of several inflammatory and anti-inflammatory proteins in the lungs may be related to the pathogenesis of asthma, and Apo A1 may be useful in therapeutic strategies.

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67 kDa laminin receptor (67LR) involvement in the trafficking of normal and leukemic hematopoietic stem cells; computer aided identification of a small inhibitory molecule

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The 67 kDa laminin receptor (67LR) is a cell-surface receptor with high affinity for laminin (LM), which plays a key role in tumour progression. 67LR is also involved in normal human T lymphocytes homing and in acute myeloid leukaemia (AML), lymphoma and myeloma cell adhesion and migration.

Recently, we demonstrated 67LR involvement in granulocyte colony-stimulating factor (G-CSF)-induced mobilization of $CD34^+$ hematopoietic stem cells (HSCs), in human healthy donors. 67LR has also been shown to contribute to HSC homing to BM after transplantation, in mice. We now investigated the role of 67LR in the trafficking of leukemic $CD34^+$ HSCs and identified a 67LR inhibitory small molecule.

Flow cytometric analysis showed a strong upregulation of 67LR expression in BM as well as in peripheral blood (PB) $CD34^+$ cells from acute myeloid leukemia (AML) patients, as compared to normal BM and PB $CD34^+$ cells. Then, we investigated whether 67LR upregulation could modulate $CD34^+$ leukemic cell adhesion and migration to LM and stromal derived factor 1 (SDF1), the key chemokine in HSC trafficking. 67LR-transfected $CD34^+$ cells from the KG1 cell line showed increased migration toward LM and SDF1, as compared to untransfected cells; on the contrary, 67LR overexpression did not increase $CD34^+$ KG1 cell adhesion to LM. 67LR activation by LM determined increased phosphorylation of p38 MAP Kinase, protein Kinase C and calcium/calmodulin-dependent protein kinase II.

Using the high resolution crystal structure of 67LR (PDB code: 3BCH), we identified 46 compounds that were predicted to interact with a previously identified LM-binding site on 67LR. Of these 46 chemical hits, compound 1-((4-methoxyphenylamino)methyl) naphthalen-2-ol, termed NSC 47924, specifically inhibited 67LR-mediated cell adhesion, migration and proliferation to LM.

Leukemic cells undergo changes in adhesive properties that allow their migration into the circulation, leading to development of extramedullary disease. Our data show that 67LR overexpression is associated with a migratory phenotype both in cytokine-stimulated normal $CD34^+$ HSCs and in leukemic $CD34^+$ HSCs. Moreover, we demonstrated that 67LR function can be specifically blocked by a newly-identified small molecule, making 67LR a promising therapeutical target.

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Urokinase receptor (uPAR) involvement in hematopoietic stem cells homing and engraftment to bone marrow

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Urokinase (uPA) is a serine protease that activates plasminogen to plasmin and binds a specific cell-surface receptor, uPAR. uPAR traditional role was considered the focusing of uPA activity on the cell membrane. However, proteolysis-independent uPAR activities have also been demonstrated. uPAR binds vitronectin (VN), interacts with various integrins and regulates their activity, mediates uPA-dependent cell migration and is required for chemotaxis induced by fMet-Leu-Phe (fMLP), a potent leukocyte chemoattractant. Soluble forms of uPAR (suPAR) have been detected in human plasma and urine. A cleaved form of suPAR (c-suPAR), lacking the N-terminal domain and exposing the sequence SRSRY (aa 88-92), stimulates cell migration by activating fMLP receptors.

We recently demonstrated uPAR involvement in G-CSF-induced $CD34^+$ hematopoietic stem cell (HSC) mobilization. We also demonstrated that c-suPAR could induce mobilization of hematopoietic stem/progenitor cells in mice.

We now investigate whether uPAR and its ligands could play a role in regulating $CD34^+$ HSC interactions with the bone marrow (BM) stroma, thus also contributing to HSC homing and engraftment to the BM.

We found expression of uPA and VN in cultures of human BM stroma cells. Interestingly, stroma cells also produced suPAR and high amounts of c-suPAR, exposing the chemotactic SRSRY sequence.

The role of the different soluble forms of uPAR produced by stroma cells in regulating HSC interactions with the BM microenvironment was analyzed by long term cultures (LTC) of BM and G-CSF mobilized $CD34^+$ HSCs, in the presence of suPAR or the uPAR-derived $uPAR_{84-95}$ peptide, corresponding to the active site of c-suPAR. Both suPAR and the $uPAR_{84-95}$ peptide increased the number of adherent and released clonogenic progenitors from LTC of BM and G-CSF mobilized HSCs.

To elucidate the mechanism of suPAR and c-suPAR effects on $CD34^+$ HSC interactions with the stromal microenvironment, *in vitro* adhesion and proliferation assays were performed on $CD34^+$ KG1 cells. suPAR treatment determined a significant increase in $CD34^+$ KG1 cell adhesion whereas c-suPAR increased cell proliferation.

Taken together, our results indicate that BM stroma produces soluble forms of uPAR that regulate $CD34^+$ HSC interactions with BM microenvironment, their local proliferation and trafficking from and to BM.

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Expression of antimicrobial peptides in patients with COPD and detection of RSV or Influenza A

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Background: The role of respiratory viruses is increasingly recognized in the pathogenesis and exacerbations of COPD. The innate immune represents the first line of defense against pathogens. Antimicrobial peptides are important effector molecules in this system.

Methods: We investigated the expression of alpha- (HNP 1-3) and beta-(h-BD2) defensins in induced sputum of COPD patients and

correlated these findings to quantitative detection of respiratory syncytial virus and Influenza A.

Results: Preliminary results show that expression of defensins was different according to the virus type detected. In patients with acute exacerbation of COPD and detection of RSV defensin levels were decreased (p=0,042) whereas in patients with acute exacerbation and detection of Influenza A there was a trend to increased defensin levels (p=0,062). Interestingly in patients with stable COPD and viral detection there was a trend to decreased defensin levels (p=0,051).

Conclusion: The differential expression of defensins could indicate different roles of different viruses. Higher expression in patients with detection of Influenza A suggests increased inflammatory response to a virus which causes virulent infection. Decreased defensin levels in case of patients with detection of RSV might support the hypothesis that RSV elicits latent infections with decreased local immune defense. Further experimental studies are warranted to better understand the complex interaction of acute and latent viral infection in COPD and the local immune response.

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Der p 1 exerts adjuvant function for Der p 2: Acute dermatitis in atopic and non-atopic mouse model

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Background: Major allergens Der p1 and Der p2 from house dust mite are important inducers of atopic dermatitis, possibly due to special molecular features: Der p1 has a cysteine protease function and may thus sensitize by disrupting the skin barrier, Der p2 may interact with TLR4 and support LPS-mediated Th2 responses.

Objective: We investigated whether Der p1 could act as adjuvant by supporting sensitization to other mite allergens. *Pichia pastoris* was thus selected as LPS-independent expression system for mite allergens Der p1 and Der p2. Imitating natural exposure in a mouse model, the two recombinant allergens were applied via the skin without adding adjuvants. Further, to examine the role of a pre-existing Th2 bias, we used in parallel atopic BALB/c and non-atopic C57BL/6 mice.

Methods*:* For sensitization mice were shaved on the back and the allergens, Der p1 or Der p2, Der p1 *and* Der p2, or PBS repeatedly applied percutaneously. The allergic status was monitored by itching score, and by the determination of Th1 and Th2 antibodies and cytokines in ELISA. Skin biopsies were taken and the histological sections were Giemsa and HE-stained. The epidermal permeability was studied by immunfluorescence staining of tight junction component ZO-1 in the biopsies.

Results: In the course of sensitizations cutaneous inflammatory lesions appeared in all Der p1, and Der p1 plus Der p2-treated mice, but not in mice treated with Der p 2 only. In both mouse strains, the Der p1-treated groups showed increasing levels of specific IgE, IgG1 (but no IgG2a) and elevated Th2 cytokine IL-5. In the biopsies from the Der p1-treated mice, eosinophil and mast cell numbers increased, the epidermis was thickened, in C57BL/6 additionally hyperpigmentated. Importantly, Der p2 was only capable to sensitize in the presence of Der p 1. ZO-1 stainings of dermatitis biopsies suggest that Der p1 indeed disturbs skin barrier function.

Conclusion: Based on our novel animal model we conclude that Der p1 allergen, repeatedly applied by the percutaneous route, can promote acute allergic dermatitis. It promotes sensitization to other Der p allergens independent on atopy and thus acts as adjuvant.

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The roles of phosphoinositide 3-kinase gamma for the pathogenesis of asthma and eosinophil functions

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Introduction Idiopathic pulmonary fibrosis (IPF) and other pulmonary fibrotic diseases induce significant morbidity and mortality, while our therapeutic options are limited. These diseases may present with inflammation early in their course, but later on are characterized by loss of lung elasticity and fibrosis with collagen deposition. Understanding the pathophysiology of these changes is a priority. Rac2 is a *ras*-related GTPase expressed primarily by inflammatory cells. Rac2 regulates a diversity of neutrophil and macrophage functions. We are interested to identify the role of Rac2 in pulmonary fibrosis. The antineoplastic antibiotic bleomycin has been used in rodents to induce lung disease similar to pulmonary fibrosis. The progression of bleomycin-induced pulmonary fibrosis includes an inflammatory phase characterized by neutrophil infiltration of the lung parenchyma followed by a fibrotic phase.

Hypothesis Rac2 deficiency protects mice from bleomycin-induced pulmonary fibrosis.

Methods C57BL/6$^{+/+}$ (wild type) and Rac2$^{-/-}$ female mice will be instilled bleomycin sulphate (1.25 U/Kg) or saline intratracheally. Twenty one days after the intratracheal instillation we will measure lung function using FlexiVent® Systems (Scireq, Montreal, QC). After lung function measurements, the left lung will be removed, fixed and then paraffin embedded. Tissue sections will be stained with Hematoxylin and Eosin (H&E) and Masson Trichrome.

Results Rac2$^{-/-}$ mice showed significantly lower mortality (30%) than wild type mice (75%) over the 21 days of the experimental protocol. Rac2$^{-/-}$ mice receiving bleomycin showed no changes in lung resistance or elastance compared to saline treated mice, while wild type mice treated with bleomycin showed increased resistance and lower elastance compared to wild type mice treated with saline. Histological analysis showed that Rac2$^{-/-}$ had decreased severity of fibrosis compared to wild type mice. Rac2$^{-/-}$ mice showed inflammatory infiltrates rather than fibrotic lesions, and had only patchy collagen deposition in the lungs compared to wild type mice.

Conclusion Rac2 is an important signaling molecule leading to lung and/or alveolar inflammation, which is important for the development of pulmonary fibrosis after administration of bleomycin. Rac2 may be an important therapeutic target in early stages of pulmonary fibrotic diseases.

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CD8 T cells stimulate dendritic cell interleukin 12 and inhibit airway eosinophil recruitment and lung inflammation by IFN-gamma independent and dependent mechanisms

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Allergic inflammation of the airways causes changes in the lung wall that lead to inflammatory diseases such as asthma.

Using a mouse model this response can be divided into an induction phase, in which CD4 Th2 T cells specific for airborne allergens are produced, and an effector phase, during which they are recruited to the lung where they orchestrate the inflammatory response marked by eosinophilia, mucus hyper secretion and increased airway hyperresponsiveness (AHR). Previously we, and others, have shown that transfer of CD8 T cells inhibits the induction of the Th2 response. In this study we have investigated the effect of CD8 T cells on the effector phase of the inflammatory lung response. *In vitro* activated OT-I CD8 T cells were transferred to ovalbumin (OVA)-alum immunized mice one day before the first of 3 airway challenges with OVA. Eosinophil infiltration was inhibited by transfer of $CD8^+$ T cells from 36.7%±4.1% to 17.6%±2.7%. When IFN-gamma$^{-/-}$OT-I crossbred mouse CD8 T cells were transferred, the inhibitory effect on eosinophilia was abolished (39.6%±5.1%), suggesting an important role for IFN-gamma produced by CD8 T cells. The importance of IFN-gamma in the suppression of eosinophilia was further demonstrated in IFN-gamma$^{-/-}$ mice which produced a greater eosinophilia (71.4%±3.2%) when sensitized and challenged with OVA. In these animals IFN-gamma-producing OT-I CD8 T cells again efficiently suppressed eosinophilia induced by OVA (28.5%±4.6%), while IFN-gamma$^{-/-}$ OT-I CD8 failed to do so (63.2%±3.4%). We also investigated the effect of CD8 T cells on lung dendritic cell (DC) function. Resident lung DCs ($CD11c^+CD11b^+CD103^-$) from CD8 transferred mice increased from 2.9×10^5 to 12×10^5 and secreted higher levels of IL-12p70, regardless of IFN-gamma. These results suggest that in addition to regulating the induction of the allergic immune response, CD8 T cells can subsequently divert the local lung environment to one that favors Th1 immunity, requiring IFN-gamma and CD8-DC interaction.

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Role of Rac2 in bleomycin-induced pulmonary fibrosis

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Introduction: Idiopathic pulmonary fibrosis (IPF) and other pulmonary fibrotic diseases induce significant morbidity and mortality, while our therapeutic options are limited. These diseases may present with inflammation early in their course, but later on are characterized by loss of lung elasticity and fibrosis with collagen deposition. Understanding the pathophysiology of these changes is a priority. Rac2 is a *ras*-related GTPase expressed primarily by inflammatory cells. Rac2 regulates a diversity of neutrophil and macrophage functions. We are interested to identify the role of Rac2 in pulmonary fibrosis. The antineoplastic antibiotic bleomycin has been used in rodents to induce lung disease similar to pulmonary fibrosis. The progression of bleomycin-induced pulmonary fibrosis includes an inflammatory phase characterized by neutrophil infiltration of the lung parenchyma followed by a fibrotic phase.

Hypothesis: Rac2 deficiency protects mice from bleomycin-induced pulmonary fibrosis.

Methods: C57BL/$6^{+/+}$ (wild type) and Rac2$^{-/-}$ female mice will be instilled bleomycin sulphate (1.25 U/Kg) or saline intratracheally. Twenty one days after the intratracheal instillation we will measure lung function using FlexiVent® Systems (Scireq, Montreal, QC). After lung function measurements, the left lung will be removed, fixed and then paraffin embedded. Tissue sections will be stained with Hematoxylin and Eosin (H&E) and Masson Trichrome.

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Conclusion: Rac2 is an important signaling molecule leading to lung and/or alveolar inflammation, which is important for the development of pulmonary fibrosis after administration of bleomycin. Rac2 may be an important therapeutic target in early stages of pulmonary fibrotic diseases.

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Mimotope therapy reduces grass pollen-induced asthma in a physiologically relevant mouse model

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Background: Mimotopes, being B-cell epitope mimics, are devoid of T-cell epitopes and thus possibly useful tools for asthma therapy. The aim of this study was to challenge this hypothesis in an experimental mouse model, mirroring the pathophysiology of human disease. Taking grass pollen allergen Phl p 5 as an example, we sensitized and aerosol-challenged mice to induce acute asthma and applied Phl p 5 mimotopes for therapy.

Methods: Acute allergic asthma was induced by systemic sensitizations and aerosol challenges with Phl p 5. For proving physiologically relevant asthma in these mice, CO_2 production and O_2 consumption during acute asthma were monitored using metabolic cages. Further, arterial blood samples were collected and subjected to blood gas analysisOne asthmatic mouse group was treated by subcutaneous administrations of the KLH-coupled peptide mimotope. Control groups received Phl p 5, irrelevant allergen, KLH alone or remained naive. The therapeutic effects of different treatments were finally evaluated after aerosol challenges with the allergen. Sera, bronchial lavage fluid (BAL) and lungs were analyzed for effects on the humoral and cellular immune response.

Results: Upon nebulisation of sensitized mice with the specific allergen (but not with a control antigen), acute significant hyperventilation with elevated CO_2, reduced O_2 levels in the exspirium and, complementarily, reduced CO_2 in the arterial blood were observed. These physiological parameters were accompanied by mucus hypersecretion and accumulation of eosinophils in the lung. However, metabolic acidosis was observed in specifically and non-specifically challenged mice, pointing towards asthma-associated bronchial hyperreactivity. When other groups of sensitized asthmatic mice were treated with the mimotope, IgE and IgG1 antibody titres remained unchanged, but the animals were protected to a high degree from bronchial eosinophilic inflammation and mucus hypersecretion upon allergen challenge.

Conclusion: The used mouse model of acute asthma is physiologically relevant and suitable for studying the

pathophysiology of allergen-induced asthma versus bronchial hyperreactivity. In accordance with our working hypothesis, Phl p 5 mimotope treatments indeed reduced the hypersecretion and inflammatory infiltrates in the lung. Therefore, we suggest mimotopes to have therapeutic potency in allergen-specific asthma.

POSTER SESSION 4: Allergy Diagnosis *In Vitro* and *In Vivo*

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Anaphylaxis to soy products in patients with birch pollinosis: Is it Gly m 4 or not?

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Objective: It was our aim to identify soy allergen(s) involved in severe systemic reactions upon first exposure to soy drinks in patients with birch pollen allergy. It is generally accepted that the soy homologue of Bet v 1, Gly m 4 is responsible for this clinical presentation.

Methods: Six birch pollen allergic patients with severe reactions to soy drinks were included. Specific IgE to birch pollen, soy and rGly m 4 were measured by SPT and/or RAST. Immunoblotting with soy-drink extracts and rGly m 4 was used to investigate whether Gly m 4 is indeed instrumental in this typical birch pollen associated soy allergy. A rabbit polyclonal antiserum against Gly m 4 was used for identification of Gly m 4 on immunoblot.

Results: All six patients with severe anaphylactic reactions after first exposure to soy drinks had a positive SPT and RAST to birch pollen, and 5/6 to soy. All demonstrated specific IgE to Gly m 4, ranging from 1.05 to 21.3 kU/L in RAST but only weakly confirmed on immunoblot with rGly m 4. Surprisingly, immunoblot analysis with soy drink did not result in IgE detection of a 17 kDa band, the expected MW of Gly m 4, despite its presence being confirmed using a specific rabbit antiserum. In contrast, IgE binding on soy drink immunoblot was dominated by doublets at ~65 kDa and at ~40 kDa and a single band at 20 kDa, all coinciding with molecular masses reported for various subunits of the soy storage proteins Gly m 5 and Gly m 6. None of these bands were influenced by inhibition with rGly m 4, suggesting that this allergen may in the end not play an important role in severe soy reactions of patients with birch pollen allergy.

Conclusions: Although Bet v 1 Gly m 4 cross reactivity is generally accepted as being at the basis of severe soy allergy in birch pollen allergic patients, our laboratory findings demonstrate that this may be an oversimplification. It may even be that the Gly m 4 reactivity is just an epiphenomenon, and that the true causative allergen still needs to be identified. This is currently being investigated.

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Peanut allergen components in relation to DBPCFC to peanut in children with suspected peanut allergy.

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Rationale: Double blind placebo controlled food challenge (DBPCFC), the existing golden standard for diagnosing food allergy, is both time-consuming and potentially dangerous. We have related peanut allergen components and basophil allergen threshold sensitivity (CD-sens) to the outcome of DBPCFC in children with suspected peanut allergy.

Methods: DBPCFC was performed with increasing concentrations of peanut allergen (1 mg to 5 g of peanut) in 16 children with suspected IgE-mediated peanut-allergy. At the challenges blood samples were taken for evaluation of CD-sens and quantification of IgE- and IgG4-antibodies to peanut and Ara h 2. Basophils were stimulated in vitro with peanut allergen and Ara h 2 in descending doses until the threshold sensitivity was reached. CD-sens was defined on the basis of the allergen dose giving 50% of maximal basophil response, measured as expression of CD63.

Results: Of 16 children 5 reacted with clinical allergic symptoms on DBPCFC. Absolute IgE antibody levels to peanut were 26.4-134 kU_A/L and the IgE antibody fraction, (% IgE antibody to peanut of total IgE), varied between 9.5-26.0 %. Remaining 11 children had low levels of IgE antibodies 0.1-1.3 kU_A/L, and did not react on DBPCFC. Children with positive challenge presented reacting basophils after stimulation in contrast to none of the children with negative challenge.

Conclusions: Peanut allergen components and CD-sens seem to correlate with DBPCFC to peanut and could be a complement/substitute to DBPCFC and thereby save patients from cumbersome clinical procedures as well as save time and money for the medical system.

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A novel lipid-transfer protein of Apium graveolens as potential cross-reactive allergen involved in the celery-mugwort-spice syndrome

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Background: Patients reacting to *Artemisia vulgaris* often demonstrate a sensitization to celery extract, which is referred to as celery-mugwort-spice syndrome. Since the underlying cross-reactive molecule in these patients was not conspicuously determined we sought to identify the respective allergen.

Methods: Mass-spectrometry based analysis, cDNA and 5'-RACE cloning was used to obtain the full-length sequence of Api g 2, a novel lipid-transfer protein of celery stalks. Recombinant Api g 2 was produced in *E. coli* Rosetta-gami B(DE3)pLysS while the natural preparation was purified from *A. graveolens* stalks. Both preparations were compared regarding their physico-chemical and immunological properties using sera of LTP-sensitized patients.

Results: A full-length cDNA sequence was identified which is in complete accordance with the natural Api g 2 consisting of a single isoallergen. Proteins were purified using ion exchange chromatography and purity was demonstrated in SDS-PAGE and mass analysis. Circular dichroism measurements revealed an identical secondary structure of the natural and recombinant protein. Both proteins showed a high thermal stability and demonstrated complete refolding capacities under acid conditions. Natural and recombinant Api g 2 showed an equivalent IgE reactivity as determined in ELISA and mediator release assays. A frequent sensitization (34/37) to Api g 2 was observed among sera from LTP-reactive Mediterranean patients. IgE cross-inhibition of celery LTP to Art v 3 and Pru p 3 was 68% and 29%, respectively as determined in ISAC inhibition assays.

Conclusions: Api g 2, a novel allergen of celery stalks demonstrates a high degree of IgE cross-reactivity with Art v 3, the lipid-transfer protein of *Artemisia vulgaris*. Thus, we presume that these heat-stable allergens might be the underlying molecules involved in the celery-mugwort-spice syndrome.

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Evaluation of diagnostic potential of 1,3-beta-glucanace from banana fruit

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Introduction. β-1,3-Glucanases are widespread proteins in higher plants, involved in various physiological processes and defense. They belong to the group 2 of the pathogenesis related (PR) protein superfamily. IgE reactive β-1,3-glucanases have been characterized from olive pollen (Ole e 9), latex (Hev b 2), and bell pepper. Due to the structural homology with Hev b 2, β-1,3-glucanase from banana fruit has been identified as potential IgE reactive protein, however its diagnostic potential has not been studied in detail.

Aim. To explore IgE reactivity of β-1,3-banana glucanase in a group of twenty two persons with symptoms of type I sensitivity to banana fruit.

Methods. Homogeneity of β-1,3-glucanase isolated from banana fruit was examined by 1D-, 2D-PAGE and 2D blot. Identity of the protein was confirmed by Edman degradation and mass fingerprint. Persons with suggestive IgE reactivity and/or positive skin prick test and ImmunoCAP to banana fruit entered the study. IgE reactivity of banana glucanase was screened in ELISA and Western blot with the patient's sera. Glucanase potential for activation of effectors cells was tested in basophil activation assay.

Results. Banana glucanase, 32 kDa protein, revealed two spots in 2D-PAGE, which shared the same N-terminal sequence (IGVXYGMLGNNL) of mature β-1,3-glucanase (NCBI AAB82772). By mass fingerprint of the isolated protein 78% of glucanase primary structure was confirmed. Fifteen out of twenty two sera (68%) revealed IgE reactivity to glucanase in ELISA. In Western blot analysis 14 out of 22 sera (63%) showed IgE reactivity to glucanase. Both glucanase isoforms, resolved by 2D blot, showed comparable IgE binding from four tested patients´ sera. Glucanase was able to activate basophils by upregulation of CD63 and CD203c molecules. More that 27% of basophils were activated with the allergen, compared with 32% and 4% of activated cells with positive and negative controls, respectively.

Conclusion. β-1,3-glucanase is an important banana allergen with a high prevalence in IgE reactivity in the studied population of patients´ sera. Isolated glucanase reveals the potential for activation of effectors cells involved in elicitation of clinical symptoms of type I hypersensitivity, and should find an application in the component-resolved diagnosis of banana allergy.

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The interaction between monoclonal antibodies and recombinant and natural purified Bet v 1

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Rationale: Monoclonal antibodies (mAbs) are often used to characterise and quantify protein entities. However, the use of mAbs requires a detailed knowledge of both affinity and specificity. This is particular important if more that mAb is used. Also the heterogeneity of the natural protein with regard to isoform may strongly influence the suitability of a quantification or characterisation assay.

Methods: We have investigated the mAb protein interaction by Biacore T100 estimating the genuine concentration of the antibody recognisable part of the protein by Calibration Free concentration Analysis (CFCA) in connection of determination of antibody protein interaction parameters and thermodynamics properties. The analysis was performed using three different mAbs (A, B and C). Iso allergen variations were explored by preparative IEF. The absolute concentration of the protein samples were determined by amino acid analysis (AAA).

Results and Discussion: The CFCA concentration of n and r Bet v 1 differed considerable from the concentration determined by AAA, indicating that the isoforms variation in the nBet v 1 strongly influence the CFCA determined concentration. Since only a part of the isoforms contained the epitopes that binding to either of the antibodies (A, B and C). However rBet v 1 is only one isoforms and it is expected the AAA and CFCA would amount to the same. The results indicate a difference in the concentration suggesting that other mechanism may be involved. We have previously observed that rBet v 1 may exist in at least two folding variants which may explain the differences observed. Further the binding kinetics and thermodynamics strongly indicate differences in the antibodies interaction with the allergens.

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Can Ig E-measurement replace challenge tests in allergic rhinoconjunctivits to grass pollen?

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Background: There is few data concerning dose-response relationship of allergen exposure and clinical reactivity for outdoor aeroallergens, such as timothy grass pollen. Timothy pollen-specific

IgE determinations might assist in predicting the clinical reactivity in patients with allergic rhinoconjunctivitis (ARC).

Objectives: Allergen-specific immunoglobulin E antibody (sIgE) levels of timothy grass pollen were correlated with individual threshold doses eliciting allergic reactions in skin, conjunctival and nasal provocation tests.

Methods: Skin prick test (SPT), nasal (NPT) and conjunctival provocation (CPT) tests were performed in patients suffering from pollen induced rhinoconjunctivitis and in healthy controls. The clinical outcomes were correlated to allergen-specific IgE antibody determinations to timothy pollen antigens and the ratio between timothy specific IgE and total IgE.

Results: One-hundred-and-forty subjects were evaluated, 104 patients with ARC (age median: 27 years; range: 18-64; females: 58 %) and 36 controls (25 years (22 - 77); females: 70 %). 96 respectively 57 percent of the patients showed a positive reaction in the nasal or conjunctival provocation. For both provocation protocols, a correlation between the dilution level at the reaction and the level of sIgE for timothy could not be established. Regarding the titrated SPT, 98 percent of the patients showed a positive skin test reaction; correlating with the level of sIgE for timothy. The ratio of sIgE/total IgE correlated with the dilution level of SPT and CPT, respectively.

Conclusion: In our study a dose response relationship between the levels of specific IgE and clinical outcome of timothy allergen exposure could not be established. However, there was a significant positive correlation between the ratio between sIgE-timothy and total IgE and the outcome of the CPT but not the NPT. Although IgE-determination remains an important key element in allergy diagnosis, provocation tests are procedures of choice if the clinical relevance of an allergen has to be evaluated.

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Characterization of the T cell response to Amb a 1, the major ragweed pollen allergen and the cross-reactivity to its mugwort homologue Art v 6

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Background: Ragweed and mugwort, both members of the *Asteraceae* plant family with overlapping pollen seasons, are the most common weeds causing pollen allergy and frequently clinical cross-reactivity is observed. Differential diagnosis between ragweed and mugwort pollen allergy based on seasonal symptoms or clinical criteria as well as differentiation between cross-reactivity and actual co-sensitization in patients with concurrent ragweed- and mugwort pollen-hypersensitivity is difficult. 95% of ragweed pollen-allergic individuals are sensitized to the major allergen in ragweed pollen, Amb a 1. Art v 6 represents the Amb a 1-homologue in mugwort pollen.

Objective: The aim of this study was to characterize the T cell response to Amb a 1 and investigate its cross-reactivity with Art v 6.

Methods: Allergen–specific T cell lines and T cell clones were established from a large number of patients allergic to ragweed- and/or mugwort-pollen. T cell epitopes of Amb a 1 and Art v 6 were determined by using synthetic 12-mer peptides for stimulation. Cross-reactivity was tested in proliferation assays and cytokine production was assessed by ELISA.

Results: The T cell response to Amb a 1 is Th2-dominated and directed against two major (Amb a $1_{178-189}$, Amb a $1_{202-216}$) and multiple minor T cell epitopes. HLA-DR, -DP and DQ molecules were found to be involved in HLA-restriction of Amb a 1 T cell epitopes. Art v 6 was found to contain only 5 relevant T cell epitope regions and Amb a 1-specific T cells cross-reacted only with a few Art v 6-derived peptides. Amb a 1 was generally a much stronger stimulus than Art v 6, indicating that Amb a 1 is the original sensitizing agent. **Conclusion:** The T cell cross-reactivity of Amb a 1 and Art v 6 was found to be limited, in line with the restricted cross-reactivity that had been observed at the IgE-level. Similarily mugwort pollen-allergic individuals have previously been found to mainly recognize Art v 1. Thus, patients sensitized to ragweed and mugwort pollen, are rather co- than cross-sensitized, indicating that specific immunotherapy in these individuals should be performed with both allergens.

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Pilot study on lupine allergy: Cross-reactivity among legumes with and without co-existing legume allergy

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Allergy to lupine, a legume and substitute in foods for wheat and soy, was in Germany first reported in 2007. A multicentre study was initiated to investigate the prevalence as well as individual patient's sensitization profiles with regard to the identification and characterization of single lupine allergens, of which only one has been described so far. Cross-reactivity to other legumes, e.g. peanut and soybean, with and without clinical relevance was observed. ImmunoCAP analysis using extracts and single recombinant allergens of these two legumes (rAra h 1, 2, 3/4, 8, 9, and Gly m 4) in addition to lupine seed extract and lupine pollen formed the initial experimental step. Protein-, DNA- and EST-data bases were queried for lupine proteins homologous to already known legume allergens. Sera were collected from 7 lupine-allergic patients with angioedema, urticaria, oral allergy syndrome and dyspnoea following lupine consumption. 2/7 suffered from co-existing peanut-allergy. None was soy-allergic. 5/7 had additional inhalant but no pollen-associated food allergies. Concomitant IgE-reactivity with peanut and soy extracts was common, the latter without clinical relevance. Only the peanut-allergic individuals had sIgE to rAra h 1, 2, 3/4. 3/7 patients had sIgE against lupine seed, peanut- and/or

soybean-extract but none against the single peanut allergens which suggests - aside from technical problems such as loss of activity of the solid phase-bound allergen - the presence of yet unknown, cross-reactive allergens in the three legumes. Additional 14 lupine-sensitized individuals were investigated, 7 had pollen-associated food allergy, 2 were soy- and another 2 peanut-allergic, basically showing similar IgE-binding patterns. ImmunoCAP analysis does not seem to uncover the whole range of potential cross-reactivity between lupine and peanut even with a large spectrum of single peanut allergens. So far, *in silico* analysis identified lupine proteins homologous to the soy and peanut allergens Gly m 3, 4, Ara h 1, 3/4, 5 and 8 as well as Len c 1 (lentil), Pis s 1 (pea), and Vig r 1 (mung bean). Cloning them and studying natural lupine allergens by proteomics will provide information indispensable for the understanding of the relevance of cross-reactivity and improve diagnosis of legume allergy.

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Allergenic and immunotoxic activities of fungi

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Several fungal species are known to induce severe respiratory allergic diseases. Fungus-induced airway obstruction is mainly associated with more severe asthma. The exact prevalence of fungal allergies is not known. Apart from their direct allergenic effects, fungi may carry mycotoxins in their spores or produce volatile metabolites. Trichotecene mycotoxins are a group of structurally toxins produced mainly by Fusarium fungi found on many crops. A variety of Fusarium fungi produce a number of different mycotoxins of the class of trichothecenes. Three of the better known toxins are T-2, HT-2 toxin and deoxynivalenol (DON, vomitoxin). The goal of this investigation was to evaluate the prevalence of fungal sensitization in patients with allergic asthma and perennial rhinitis as well as to analyze the immunotoxicity and the role of Trichotecene mycotoxins in allergy. The sensitivity of 32 patients with mild, moderate and severe asthma combined with allergic rhinitis has been studied by skin prick tests (SPT) using different fungal allergens - Alternaria tenuis, Candida albicans, Aspergillus fumigatus, Hormodendrum cladosporioides, Fusarium vasinfectum (Hollister-Stier Lab., USA) and Cephalosporium (Antigen Lab., USA) as well as with house dust mite allergens -D. farinae and D. pteronyssinus (Biomed, RF). 21 among of all studied patients (65.6%) had positive SPT to house dust mite allergens. Among 32 patients only two (6.25%) had no sensitization to all investigated fungi allergens. In these two patients the mild asthma has been diagnosed. Five severe asthma patients (15,6%) were sensitized to all studied fungal allergens. The SPT demonstrated that the sensitivity of asthma patients to different fungi ranged as following: Alternaria tenuis -56,3%, Candida albicans -50,0%, Aspergillus fumigatus - 46,9%, Hormodendrum cladosporioides -37,5%, Cephalosporium -21,9%, Fusarium vasinfectum -15,6%. In experimental study it has been shown that T-2 toxin and DON dose-dependently increase synthesis of anti-ovalbumin-IgE-Ab and this effect was not genetically restricted. Mycotoxins increased an IgE synthesis in the culture of PBMC of healthy donors as well as in allergic asthmatics.Both mycotoxins dose-dependently inhibited stem cell proliferation in mice and PHA-induced MNC proliferation. T-2 toxin and DON suppress the activity of peritoneal, spleen, bone marrow and alveolar macrophages. Thus, allergic asthma and rhinitis induced by house dust mite allergens frequently associated with the sensitization to different fungal allergens. Fungal allergen exposure may be one of the important factors increasing the severity of asthma and immune disturbances in allergic disease.

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IgE-mediated Diclofenac allergy: Induction mechanism and improvement of diagnosis

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Background: In a 7 years-retrospective analysis of our outpatient clinic population with drug hypersensitivity reactions to non-steroidal anti-inflammatory drugs (NSAID), diclofenac was found to be the most common culprit for severe anaphylactic reactions, suggestive for an IgE-mediated mechanism. There are two major questions standing: A) How can sensitization and IgE formation occur against this hapten and B) why is drug allergy diagnosis inconsistent?

Objective: Oral diclofenac largely binds to albumin in stomach juice thereby acquiring the features of a complete antigen. In analogy to the development of food allergies, we aimed to investigate whether co-medicated anti-ulcer drugs could explain the underlying pathomechanism for IgE-mediated drug sensitization. Second, diagnosis is today based on intradermal tests with monovalent diclofenac, not considering that IgE crosslinking is required for mast cell activation. We thus proposed that a multivalent diclofenac conjugate could improve drug allergy diagnosis.

Methods: A) Diclofenac was orally applied to BALB/c mice with or without gastric acid suppression, alone or coupled to mouse serum albumin. Sera were tested in ELISA and rat basophilic leukemia test, and mice subjected skin tests. B) In two of our patients with generalized urticaria to diclofenac and two control persons, we performed skin prick tests (SPT) and ELISA for IgE antibodies using diclofenac coupled to keyhole limpet hemocyanin (KLH), uncoupled KLH or diclofenac.

Results: A) Only mice receiving albumin-coupled diclofenac under gastric acid

suppression developed anti-diclofenac IgG1 and IgE in a dose-dependent manner.

The induced antibodies triggered mast cell degranulation and positive skin tests.

B) IgE antibodies in patients´ sera towards diclofenac could be detected in ELISA

coated with the diclofenac-KLH conjugate. Further, the diclofenac-KLH conjugate elicited wheal and flare reactions, whereas KLH alone or diclofenac (even at a 100 times higher concentration) did not elicit skin prick reactivities.

Conclusion: A) Gastric acid suppression is suggested a causative mechanism in the induction of IgE-mediated diclofenac allergy. B) Testing with diclofenac-KLH conjugate improved diagnosis of IgE-mediated diclofenac allergy.

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Abstracts

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Component-resolved analysis of IgE antibody responses in pediatric patients allergic to domestic animals

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Background*:* Domestic animals contribute to quality of life for many people but are also common causes of asthma and other symptoms of respiratory allergy among atopic individuals. Improved diagnostic procedures, including component-resolved diagnostics (CRD), may provide for more adequate guidance regarding treatment and allergen avoidance.

Objective*:* To study IgE antibody responses to allergen components from cat, dog and horse in a cohort of children and adolescents with a clinical diagnosis of allergy to one or more of these animals.

Methods*:* 157 patients allergic to cat, dog or horse and 14 pollen allergic controls were included. Clinical history and current symptoms were recorded using a standardized questionnaire. Sensitization to each allergen source was tested by SPT and ImmunoCAP. IgE antibody responses to allergen components Fel d 1-4, Can f 1-5, Equ c 1, 2, 4/5 and a novel horse dander allergen were determined by ImmunoCAP.

Results: 140 of the 157 patients were allergic to cat, 88 to dog and 70 to horse. 97 patients were allergic to 2 or more of the animals and 44 to all three. 75 of the dog allergic and 63 of the horse allergic patients were also allergic to cat. 46 patients were allergic only to cat, 10 only to dog and 4 only to horse. While extract-based tests detected IgE antibodies to cat, dog and horse in 94%, 95% and 93% of the patients allergic to each of the animals, positive results to at least one allergen component were obtained in 96%, 82% and 91%, respectively. Levels of IgE to Fel d 1 were comparable with those to cat dander. In the case of dog and horse, a more even distribution of IgE responses to different components was observed and cumulative levels to components were comparable to levels measured with extract. Low-level IgE binding to dog dander extract was detected in 4 pollen allergic controls whereas only one showed IgE binding to dog allergen components.

Conclusion*:* CRD did not provide for higher diagnostic sensitivity than extract-based tests in this study but appeared to enable higher clinical specificity.

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Lipids modify structure and digestibility of peanut allergen Ara h 8

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Peanut extract is one of the most allergenic foods consisting to about 50% of lipids. Some of its allergens are associated with lipids: the Bet v 1 homolog Ara h 8, the lipid transfer protein Ara h 9 and the oleosins Ara h 10 and 11. We wanted to study, how the Ara h 8 molecule interacts with lipids and whether this interaction might have an impact on allergenicity.

As a Bet v 1 homolog, Ara h 8 is assumed to form a hydrophobic pocket, to which lipids can bind. This was verified by a displacement assay using the fluorescent stain ANS, which bound to the cavity of recombinant Ara h 8 (rAra h 8) and rBet v 1. The affinity of Ara h 8 to lipids was further confirmed by replacement experiments after preloading rAra h 8 with natural peanut oil. The binding of lipids to rAra h 8, peanut oil-preloaded rAra h 8 and natural Ara h 8 isolates was further analysed with different types of lipids. Aliphatic lipids (stearic acid, oleic acid, linoleic acid) were bound with a higher affinity than cholinergic lipids (deoxycholate, cholesterol). Since peanut oil contains considerable amounts of linoleic acid (13-35%), which is known as trigger for a TH2 response, lipid loading and transport by Ara h 8 might therefore have an allergy-promoting effect.

The high lipid content of peanuts may also influence the degradation of Ara h 8 by proteolytic enzymes. Ara h 8 preparations were examined for digestibility by use of gastric and pancreatic enzymes and subsequent separation by SDS electrophoresis. Pepsin degraded rAra h 8 rapidly, while the natural counterpart and the oil-preloaded rAra h 8 remained unchanged. Using a pancreatic surrogate, pure rAra h 8 was much faster degraded than the oil-preloaded rAra h 8. These results indicate a delayed degradation of Ara h 8 associated with lipids.

Our studies demonstrate that lipid binding modifies the structure and digestibility of Ara h 8, which might increase the allergenicity by a prolonged exposure to immune cells.

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X-ray crystallographic mapping of antigenic determinants in Bla g 2 using non-overlapping monoclonal antibodies

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Rationale: We previously reported the X-ray crystal structure of the mAb 7C11 with the cockroach allergen Bla g 2 showing that the epitope is involved in IgE antibody binding and located in the N-terminus of this bilobal allergen. In order to analyze the antigenic surface of Bla g 2, the structure of the allergen in complex with a non-overlapping antibody (4C3 mAb) was solved.

Methods: The structure of a partially de-glycosylated Bla g 2 mutant (N93Q, glycosylated at positions N268 and N317) co-crystallized with the mAb 4C3 was solved at 1.8Å resolution. Mutants of residues involved in the epitope, a de-glycosylation mutant (N268Q) and a monomeric mutant were expressed in *Pichia pastoris* and analyzed for antibody binding by ELISA and/or multiplex array. Affinity of 4C3 mAb binding to the allergen in the 10nM range was measured by surface plasmon resonance.

Results: The 4C3 mAb epitope is conformational involving four sections of the allergen, and is located at the C-terminal lobe of the allergen, opposite to the lobe where 7C11 mAb binds. Unlike 7C11 mAb interaction with Bla g 2, a sugar (N-acetylglucosamine at position N268) is involved in the interaction through the antibody heavy chain, and zinc and more solvent molecules are involved. An ELISA using a N268Q mutant showed that the sugar is not essential for the allergen-antibody interaction. A set of single mutants of the main amino acids involved in the epitope (E233A, E233R,

D248A, K251A) did not show a significant effect on 4C3 mAb binding. However, the mutant E233R+I199W showed a strong reduction on 4C3 mAb binding. Isolelucine 199 contributes to a hydrophobic pocket recognized by W105 from the antibody heavy chain. The relative amount of IgE antibody bound to a monomeric Bla g 2 mutant presented by non-overlapping mAbs varies between patients. This result indicates patient differences in polyclonal responses in either amount of antibody produced by epitope-specific clones or in the location of epitopes.

Conclusions: The identification of antigenic determinants on the allergen by X-ray crystallography facilitates the analysis of their involvement in IgE antibody binding for a better understanding of the allergic response.

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The very limited usefulness of skin testing with penicilloyl-polylysine and the minor determinant mixture in evaluating nonimmediate reactions to penicillins

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Background: The contribution of skin testing with penicilloyl-polylysine (PPL) and the minor determinant mixture (MDM) to the diagnosis of hypersensitivity reactions to penicillins differs greatly according to the type of reaction: immediate (i.e., occurring within one hour after the last drug administration) or nonimmediate (i.e., occurring more than one hour after the last drug administration).

Objective: To assess the contribution of skin testing with PPL and MDM to the diagnosis of nonimmediate reactions to penicillins.

Methods: We evaluated 162 adults who had had 232 nonimmediate reactions to penicillins, mostly aminopenicillins, and presented positive skin and/or patch tests to one or more penicillin reagents: PPL, MDM, benzylpenicillin, ampicillin, and amoxicillin, as well as any responsible penicillins.

Results: One hundred and fifty-seven subjects (96.9%) displayed patch-test and/or delayed-reading intradermal-test positivity to penicillin reagents, 6 also presented immediate-reading skin-test positivities. Although there were 16 patients with delayed-reading intradermal-test positivity to MDM, all 157 patients with a cell-mediated hypersensitivity were positive to the responsible penicillins (parent drugs).

Five (3.1%) of the 162 patients displayed only immediate-reading skin-test positivity (4 to PPL and 1 to amoxicillin).

Overall, 158 subjects (97.5%) presented positive responses to the responsible penicillins, while only 9 (5.5%) and 17 (10.5 %) were positive to PPL and MDM, respectively.

Conclusions: The contribution of skin testing with PPL and MDM in diagnosing nonimmediate hypersensitivity reactions to penicillins, especially cell-mediated ones, is very limited. This finding could be useful at a time when PPL and MDM are not available in all countries.

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Assessment of cut off points for pollen allergens represented in ISAC microarray CRD103

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Introduction: In recent years, a new in vitro molecular allergy diagnostic tool has been launched, but few studies on cut off point, specificity and sensitivity values have been performed.

The aim of the present study was to assess these parameters in seven of the most common pollens in our environment in pollen allergic patients.

Material and Methods: 130 patients with rhinitis, rhinoconjuctivitis or asthma and 21 non atopic controls were included. Seven pollen allergens were evaluated in all patients: *Phleum pratense*, *Olea europaea*, *Cupressus arizonica*,

Inclusion criteria consisted in subjects presenting symptoms within pollen season (related with each polen allergen) with positive skin tests and positive specific IgE to the pollen involved. We consider as controls, subjects without clinical history with pollen, and negative skin tests and specific IgE to this particular allergen.

We analysed the sensitivity and specificity for each antigen by means of ROC curves, choosing the cut-off point that offers the best specificity.

All the patients underwent specific IgE determination to allergenic components by ISAC microarray technique CRD103 (Phadia, Sweden). Seventeen pollen components were analysed (Phl p 1, 2, 4,5,6,7,11,12; Cup a 1; Ole e 1,2;).

Results: Nine out of the 11 pollen components included in our study showed significant values of area under the curve (AUC): Phl p 1,2,4,5,6,12; Ole e 1,2; Cup a 1; components.

The sensitivity, specificity and cut off values vary depending on the protein analysed. In our hands, some of the best sensitivity and specificity values are included in the following table:

	Sensitivity	Specificity	Cut off (ISU)	AUC
Phl p 1	82	97	0.3	0.9
Phl p 4	82	100	0.3	0.9
Phl p 5	77	100	0.1	0.88
Cup a 1	100	100	0.3	1

Conclusions: In general good specificity values were observed and quite good sensitivity corresponding to the semi-quantitative technique that it is.

The cut off point for the best specificity and sensitivity values could be 0.3 ISUs. For a correct application of this technique, studies evaluating the isolated behaviour of each protein are needed.

Further studies should be performed in a larger sample for all the allergens included in this microarray.

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Heparin associated dermal necrosis as manifestation of heparin induced thrombocytopenia II?

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Background: Necrotic skin lesions after application of Heparin are rare. They typically manifest between the day 6 and 12 after starting subcutaneous (s.c.) application.

Case report: A 62 year old woman was started on Enoxaparin s.c. and on Phenprocoumon orally because of heart disease. On day 7 she developed an erythemateous plaque at the injection site of Enoxaparin, which was stopped. At

presentation in our clinic, the plaque had transformed to necrosis.

Results: Laboratory investigations showed circulating antibodies against the platelet factor 4-complex (PF4-complex) but no concomitant thrombocytopenia. The Heparin – induced platelet aggregation test (HIPA) was negative as well as skin prick and patch test to Enoxaparin.Under a topical therapy the abdominal lesion showed scar healing within four months.

Discussion: The etiology of heparin necrosis is not clear. Antibodies against the PF4-complex, which acts as antigen in the immunological form of heparin induced thrombocytopenia (HIT II), are frequently found in the serum of patients with heparin necrosis. In HIT II these antibodies lead to arterial and venous thrombosis via platelet activation in half of the patients. In contrast, only a minority of patient with skin necrosis show a significant platelet drop or develop thromboembolic complications - so a HIT II cannot be diagnosed with certainty. An isolated, HIT II-antibody mediated thrombosis of dermal vessels can be suspected as underlying cause of the skin necrosis. Also, a certain traumatic factor (s.c. application?) might be necessary in order to develop necrosis. If a HIT II or a heparin induced necrosis is clinically suspected, anticoagulation should be switched to alternative agents like Lepirudin, Argatroban or Danaparoid.

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Recombinant allergens in the diagnosis of pollen-allergic patients: Comparison with natural allergens extracts

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The diagnosis of Type I allergy is based on the measurement of allergen-specific IgE antibodies and on provocation tests with allergens. Current forms of allergy diagnosis are performed with allergen extracts. The standard *in vitro* assay for measuring allergen-specific IgE does not permit the identification and measurement of specific IgE to individual allergenic components in the extract. The development of recombinant allergens offers new possibilities to study IgE reactivity profiles. Several studies have demonstrated the advantages of recombinant allergen based diagnosis. Component-Resolved Diagnosis with recombinant allergens reveals the antibody reactivity profile of allergic patients and identifies the disease-eliciting allergen molecules. The presence of IgE to cross-reactive allergen components can be determined and used to predict clinically relevant sensitization to allergen sources which contain immunologically related allergens.

The aim of our study was to demonstrate in sera of patients allergic to some common pollens the presence of IgE specific to recombinant molecular components thus enabling the development of new IgE reactivity profiles. In fact using recombinant allergens in *in vitro* diagnostic devices, a patient's individual IgE reactivity profile can be quantitatively established. We focused on allergenic recombinant components of pollen extracts such as Bet v1-v2-v4, Phl p1-p5b-p7-p12, Ole e1, Par j2, Art v1, Alt a1, Fel d1.

Blood samples of 50 patients with clinical history of pollen hypersensitivity and/or allergy were collected. We used a panel of purified natural and recombinant allergens to establish the molecular sensitization profiles. Sera were analyzed to measure total and specific IgE; IgE concentrations were determined by ImmunoCAP 250 System (PHADIA–Italy).

Preliminary data indicated a good correlation between clinical history and skin prick test *versus* specific IgE concentrations *in vitro* evaluated by a large panel of allergen natural extracts. The use of specific panel of recombinant allergens showed in all patients the presence at different concentrations of specific IgE for some recombinant molecular components; IgE sensitization was also observed against highly cross-reactive recombinant molecular components. Particularly interesting was the presence in patients allergic to Fagales (t3,t4,t7) or Graminaceae (g5,g6,g8) of IgE specific to recombinant pan-allergens as rPar j2 or rAlt a1.

The identification of sensitization profiles may play a paramount role in the choice of specific allergen-based immunotherapy and in monitoring the success of the treatment.

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Allergen sensitization of infants and 1-6 years old children in Debrecen (Hungary) within 2004-2006 years

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The aim of the investigation was to make an analysis on the actual occurrence and distribution of the dominating food and inhalative allergens in a group of 427 infants and another group of 1-6 years old 818 children, using allergen specific IgE determinations by ALLERgen in vitro diagnostic (ELISA) system.

In infants the allergic sensitization was almost totally caused by 5 food allergens (cow milk, egg white, hazelnut, wheat flour, peanut).

In 1-6 years old children the importance of indoor inhalant allergens increased as well as the number of provoking food allergens, and the dominating role of cow milk and egg white remained.

All the details of the occurrence of the different allergens are presented.

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Enzymatic characterization of house dust and storage mite allergen extracts

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There is scarce information about the biological function of many allergens in house dust and storage mites. In the last years, several studies have reported on the enzymatic activity of some mite allergens. Objectives: To investigate the enzymatic activity in whole cultures and mite bodies of house dust and storage mites. Material and Methods: Chitinase, collagenase, cysteine protease and keratinase activity was determined in extracts of *Dermatophagoides pteronyssinus* (DP), *D. farinae* (DF), *D. microceras* (DM), *Euroglyphus maynei* (EM), *Glycyphagus domesticus* (GD), *Blomia tropicalis* (BT), *Acarus siro* (AS) and *Chortoglyphus arcuatus* (CA). All mite species were grown on a low allergenic substrate. Mite

bodies were harvested using a modified escape-method. Whole cultures and mite bodies were extracted and stored at -20 °C until further use. Protein content was determined according to method of Bradford. Enzymatic assays were performed following standardized procedures. Results: Chitinase activity was detected in all extracts from whole culture and mite bodies. Chitinase activity was higher in storage than in house dust mites. The greatest activity was detected in the body extracts of storage mites. Cysteine protease activity was detected in all mite bodies; whole cultures showed little or no activity. Cysteine protease activity in mite bodies of DF, DM, and DP was much higher than in mite bodies of storage mites. Collagenase activity and keratinase-activity was detected only after prolonged incubation. Whole cultures had more collagenase activity than mite bodies. Conclusions: Cysteine protease and chitinase activity are clearly present in all extracts, but whole cultures have much less activity than mite bodies. Mite bodies from storage mites have higher chitinase activity than house dust mites. Whole cultures have more collagenase and keratinase activity than mite bodies.

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Commercial *Dermatophagoides pteronyssinus* extracts show a great variability regarding the allergen content and cutaneous responses

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Background: House dust mite extracts used for diagnosis and immunotherapy consist of a mixture of allergenic and nonallergenic components and are not well standardized.

Objective: To compare commercially available *Dermatophagoides pteronyssinus* extracts from different manufacturers regarding their allergen content and their skin test responses in *D. pteronyssinus* allergic subjects.

Methods: Rabbit antisera specific for Der p 1, 2, 5, 7, 10 and 21 were used to detect these allergens in the different extracts by immunoblotting. Quantitative ELISAs were used to quantify Der p 1 and Der p 2 in the extracts. Twenty seven mite-allergic subjects were skin prick tested in Italy with 9 different extracts and 23 subjects in France with 4 extracts. Sera from all subjects were tested for IgE-reactivity with nDer p 1, rDer p 2, nDer p 4, rDer p 5, 7, 8, 10, 14, 20 and 21 in dot blot assays.

Results: Only Der p 1 and Der p 2 were detected in all extracts but in considerably different amounts (Der p 1: 6.0µg mL^{-1} – 40.8µg mL^{-1}; Der p 2: 1.7µg mL^{-1} – 45.0µg mL^{-1}). Der p 5, 7, 10 and 21 were only detected in certain extracts and also in variable amounts. Highly differing skin test reactions were observed with the different extracts. 26% of the Italian subjects and 4% of the French were not diagnosed with certain extracts.

Conclusion: Commercially available *D. pteronyssinus* extracts lack important allergens, show great variability regarding content of certain allergens and may fail in diagnostic tests.

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Identification and characterization of a conformational epitope on the cashew nut 11S globulin allergen, Ana o 2

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The 11S globulins are members of the cupin protein superfamily and represent an important class of tree nut allergens. Although a number of linear epitopes have been mapped for many of these allergens, specific conformational epitopes have yet to be described even though conformational epitopes appear to play an important role in some food allergy responses.

The well studied cashew allergen, Ana o 2, an 11S globulin or legumin, was used to screen a set of murine monoclonal antibodies (mAb) by ELISA and one, mAb 2B5, which overlaps a patient IgE-reactive conformational epitope was identified. Treatment of native Ana o 2 with denaturing agents revealed that the 2B5 epitope is highly susceptible to denaturation by SDS, beta-mercaptoethanol, urea, and boiling. Variously constructed recombinant chimeric proteins composed of Ana o 2 and homologous segments of soybean Gly m 6 were used to demonstrate that the 2B5 epitope is expressed on the large (acidic) subunit of Ana o 2, but only when associated with an 11S globulin small (basic) subunit. Both the Ana o 2 and the homologous soybean Gly m 6 small subunits can foster 2B5 epitope expression, even when the natural N-terminal to C-terminal subunit order is reversed in chimeric molecules.

We have also fine-mapped the epitope using a combination of recombinant chimeric molecules, deletion and point mutations, molecular modeling, and electron microscopy of 2B5-Ana o 2 immune complexes. Key 2B5 epitope residues appear confined to a 24 amino acid segment near the N-terminus of the peptide, a portion of which makes direct contact with the small subunit. Molecular modeling shows that the 2B5 segment includes two beta strands on one lip of the beta-barrel and a small helical region near the small subunit. Mutation of surface-exposed residues in the region surrounding the 2B5 peptide segment on both the large and small subunits did not interfere with 2B5 binding, suggesting that the 2B5 contact residues lie within the 24 amino acid segment. The modeling data provide an explanation for both the small subunit dependence of 2B5 epitope expression and its structurally labile nature.

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Increased allergen-specific IgE titers are linked to increased diversity of the serum IgE composition

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Abstracts

Rationale: Using a panel of monoclonal anti-Der p 2 recombinant-IgEs (rIgE) we previously demonstrated that the composition of the IgE-repertoire i.e. IgE-concentration, individual IgE-specificities and individual IgE-affinities are the governing factors for activation of mast-cells/basophils through FcεRI cross-linking and activation of allergen-specific T-cells through CD23 mediated Facilitated Allergen Presentation (FAP). Similarly, the composition and degree of complexity of allergic patients' IgE-repertoire may determine the severity of allergic reactions.

Methods: The capacity of sera from Der p 2-sensitized individuals to mediate the complex-formation required for FAP was measured by FACS (CD23-IgE-Der p 2 complex-formation on CD23+ B-cells). In parallel FAP-experiments, sera were supplemented with monoclonal rIgEs that each binds to one of three non-overlapping epitopes on Der p 2. Moreover, sera collected from the same individuals in 1990 and in 1997 was analysed for their capacity to induce FAP complexes when combined with one or two high affinity rIgE molecules to investigate how serum IgE composition changes over time.

Results: Three serum-groups were recognized 1) supporting complex-formation with Der p 2 on their own, 2) supporting complex-formation when supplemented with single rIgE's, or 3) only supporting complex-formation when supplemented with pairs of rIgEs. The degree of complex-formation in the different groups correlated with affinity and/or clonality of the rIgEs added and the serum capacity for complex-formation between groups increased with increasing Der p 2-specific serum IgE-concentrations. Finally, experiments with patient sera collected twice, 7 years apart showed that increased IgE titers developed over this period resulted in increased complex formation similar to the differences between the serum groups described above.

Conclusion: IgE-composition of human sera can be indirectly assessed by combining sera with defined monoclonal IgEs in FAP-assays. The observed differences in epitope-coverage of Der p 2-specific serum-IgE between the groups indicate that increased IgE-titers correlate with increased complexity of the IgE-repertoire and diagnostics addressing this diversity may be used to predict the progression of allergic diseases.

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Component resolved diagnosis (CRD) for hazelnut allergy in EuroPrevall

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Background: Hazelnut is the most common cause of tree nut allergy in Europe. It can be pollen or non-pollen related and can give rise to severe systemic reactions. A CRD study has recently been performed in 3 European countries, but since then two new hazelnut allergens have been cloned and sequenced and preliminary data indicate these can play an important role in hazelnut allergy.

Objective: To perform a hazelnut CRD study with all hazelnut allergens known to date, on an extensive group of hazelnut allergic patients recruited at 12 clinical centres distributed across Europe.

Methods: Within a multi-centred European consortium, EuroPrevall, patients with a well-documented history of food allergy were included in 12 clinical centres. All patients were tested with ImmunoCAP (CAP) and skin prick test (SPT) to a panel of 37 food/pollen extracts and double-blind placebo-controlled challenges (DBPCFC) were performed in selected patients. Hazelnut 2S albumin (Cor a 14) was cloned, sequenced and expressed in *E. coli* and a preparation of protein enriched for natural hazelnut oleosin (Cor a 12/13) was isolated from oil bodies and analyzed by mass-spectrometry (MS). In addition, the following allergens were produced and characterized with SDS-PAGE, MS and circular dichroism (CD) spectra: rCor a 1, rCor a 2, rCor a 8 and nCor a 9.

Results: Of 573 hazelnut allergic patients included in the study, 425 have so far been tested with SPT/CAP. 306 (72%) were CAP/SPT positive (+), 37 (8%) CAP +/SPT negative (-), 20 (5%) CAP -/SPT + and 62 (15%) negative to both. 131 of the patients were subjected to DBPCFC: 90 tested positive, 24 negative and 17 were placebo reactors.

Conclusion: The availability of sera from a large number of clinically well-defined (and partly challenged) patients in combination with a panel of 7 purified hazelnut allergens, 2 pollen allergens and 4 extracts will allow us to set up an *in vitro* CRD study to assess geographical differences in symptom severity and sensitisation profile to individual purified hazelnut allergens. Additionally, putative differences in allergen recognition by young vs. older patients (primary sensitization by hazelnut/birch pollen) may be assessed.

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Identification and immunoreactivity of almond lipid transfer protein, Pru du 3

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Introduction: Plant non-specific lipid transfer proteins (LTP) have been identified as allergens in a variety of fruits, vegetables, and nuts, and several studies have demonstrated cross-reactivity among LTPs. The role, if any, of LTP in almond allergy was previously unknown.

Methods: To identify LTP in almond, polymerase chain reaction (PCR) was preformed using gene specific primers designed to amplify the gene from an almond cDNA library. The coding region of the gene was ligated into the pMal-c4X expression vector and expressed as a maltose binding (MBP) fusion protein. The fusion protein was affinity purified by passage over an immobilized amylose column and the MBP fusion tag removed by proteolytic digestion. The recombinant LTP was used to screen almond-allergic patients' sera for IgE reactivity using immunoblot, ELISA, and dot blot assays. A cross-reactive rabbit anti-peach LTP antiserum was used to identify the relevant band in a Western blot of almond extract.

Results: Native LTP was identified in the crude nut extract. IgE reactivity to almond LTP was found in 5 of 25 (20%) patients tested by dot blot assay and designated Pru du 3. Surprisingly, only 2 of the 5 reactive patients recognized the LTP band in a Western blot under reducing conditions using a low molecular weight-enriched almond extract (i.e., a LTP-enriched fraction). As Western blotting conditions typically lead to protein denaturation, the lack of IgE reactivity by

immunoblot suggests that certain patients may recognize primarily conformational epitopes on LTP. Dot blot assays demonstrated a loss of IgE reactivity to recombinant LTP in these patients when treated with common reducing reagents used in immunoblotting.

Conclusion: The results demonstrate that LTP is an allergenic protein in almond. IgE antibodies in Pru du 3-allergic individuals appear to be directed against conformationally stable and labile epitopes with certain patients recognizing only labile conformational epitopes.

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Structural method development on biological reference preparations: GMP-produced recombinant Phl p 5.0109 as a model allergen in different formulations

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Standardization of allergen extracts requires availability of well-characterized recombinant allergens used as reference standards provided by the European regulatory authorities. The major pollen allergen of *Ph. pratense*, Phl p 5.0109, has been among the first biological reference preparations being characterized in detail at the physicochemical level. This task has been pursued in frame of the Biological Standardization Program BSP090 under the auspices of the European Directorate for Quality of Medicines and Healthcare (EDQM). Recombinant Phl p 5.0109 was produced under GMP conditions and analyzed by an array of physicochemical methods. A number of characteristic parameters including identity, quantity, homogeneity, folding, denaturation susceptibility, and aggregation behavior have been verified and/or determined unequivocally. Recombinant Phl p 5.0109 was shown to represent a highly stable, well-folded, monomeric, and immunological equivalent of its natural counterpart suitable for use as a candidate reference standard for allergen products of timothy grass. Extensive structural investigations based on Fourier Transform-infrared spectroscopy (FTIR) have been performed on recombinant Phl p 5.0109 in different formulations, as this technique can be applied on proteins in solution and alum-adsorbed suspension, as well as on proteins in lyophilized state. Folding studies revealed similar secondary structure and aggregation behavior in solution, alum-adsorbates, and lyophilisates at standard conditions. However, thermal stability seemed to be influenced by different formulations, as indicated by different denaturation behaviors determined in FTIR temperature ramping experiments. Linking such structural data to IgE-binding, IgE receptor-crosslinking, and antigen processing studies interesting conclusions may be drawn on the immunological behavior under physiological conditions. Alterations in structural stability caused by protein formulation may have significant implications on therapeutic intervention. Fourier transform-infrared spectroscopy has qualified as a very versatile tool for investigating structural bahavior of allergens in different formulations.

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Peanut allergy: Clinical and immunological differences among patients from three different geographical regions

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Background: Peanut allergy is a worldwide problem, affecting people from different geographical regions with different pollen exposure.

Aim: To describe the clinical and immunological characteristics of peanut allergic patients from three countries (Spain, USA and Sweden) with low, intermediate or high grass or birch pollen exposure, using a molecular component diagnosis approach.

Methods: Peanut allergic patients from Madrid (Spain, n=50), New York (USA, n=30), Gothenburg, and Stockholm (Sweden, n=35) were enrolled. Clinical data were obtained either from a specific questionnaire or gathered from the chart reviews. IgE antibodies to the peanut allergens Ara h 1, 2, 3, 8 and 9 as well as IgE antibodies to birch (Bet v 1) and grass (Phl p 1, 5, 7 and 12) pollen allergens were analysed by the ImmunoCAP System (Phadia, Uppsala, Sweden).

Results: A total of 115 subjects were investigated. American patients frequently had IgE antibodies to rAra h 1, rAra h 2, and rAra h 3 (56.7-90.0%) and often presented with severe symptoms. Spanish patients recognized these three recombinant peanut allergens less frequently (16.0-42.0%) and were more often sensitized to the lipid transfer protein, Ara h 9 (60.0%), and typically developed peanut allergy after becoming allergic to other plant-derived foods. Swedish patients detected rAra h 1-3 more frequently (37.1-74.3%) than the Spanish patients and had the highest sensitization rate to the Bet v 1 homologue, rAra h 8 (65.7 %), as well as to rBet v 1 (82.9%). Spanish patients were more often sensitized to the important plant panallergens Phl p 7&12 (24.0%) compared to the Swedish (8.6%) and American (3.3%) patients. The Swedish patients were in equal proportions sensitized to a total of 1, 2, 3 or 4 peanut allergens (20.0-25.7%), in comparison with the American (56.7% recognized 3 allergens) and the Spanish (54.0% recognized only one allergen) patients. Spanish and Swedish patients became allergic to peanut at 2 years or later whereas the American children around 1 year of age.

Conclusions: Peanut allergy has different clinical and immunological patterns in different areas of the world. Allergen component diagnostics help us understand this complex entity.

Abstracts

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Molecular mapping of surface exposed epitopes on the Group 1 mite allergens: Towards the resolution of the cross-reactive "Heymann" epitope

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Rationale: Over 20 years ago, monoclonal antibody-based epitope mapping studies identified multiple species specific epitopes on the Group 1 mite allergens, and a unique cross-reactive epitope defined by mAb 4C1, which inhibited human IgE binding (*Heymann et al, J Immunol 1986*). Human IgE responses to mite Group 1 allergens are strongly cross-reactive and mAb 4C1 is a probe for identifying surface exposed allergenic determinants. The aim of this study was to determine the X-ray crystal structures of Group1 allergens in complex with mAb 4C1 to localize key amino acids involved in allergen antibody interactions.

Methods: High resolution crystal structures of natural Der p 1 and Der f 1, allergens in complex with mAb 4C1, and purified Fab fragments of mAb, were determined by X-ray diffraction analysis. Diffraction data were collected using synchrotron radiation (Argonne National Laboratory, Structural Biology Center) and analyzed using HKL-3000 software package.

Results: The crystal structures of Der p 1 and Der f 1 were resolved at 1.4Å and 2.0Å, respectively. The cysteine protease catalytic site in both allergens was conserved and four non-overlapping 'patches' that differed between both allergens were identified. The crystal structures of a 4C1 Fab fragment and a complex of Der f 1 with mAb 4C1 were resolved at 2.05Å and 1.9Å, respectively. Four amino acids on the surface of Der f 1 were identified as the most important contact residues for binding the heavy and light chains of the Fab region. The epitope is formed by the same amino acids in the same conformations in both Der p 1 and Der f 1. The amino acids forming the epitope have the same conformations whether complexed with antibody or not. The crystal structure of 4C1 alone shows that the CDR regions of the antibody do not significantly change in conformation upon allergen binding.

Conclusions: Definitive molecular mapping studies have identified key amino acids involved in a unique cross-reactive epitope on the Group 1 mite allergens. These amino acids could be involved in IgE recognition and have biologic and clinical significance.

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A monomeric, low IgE binding Bet v 1 derivative produced by epitope grafting provides an excellent candidate for birch pollen IT

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A safe application of recombinant allergens in specific immunotherapy is very much dependent on low-IgE binding molecules ideally showing strong T cell-modulatory properties. Computer-based analysis of the Bet v 1 family identified an epitope, which is highly susceptible to changes of the primary structure resulting in alternatively folded molecules. Using this knowledge a hypoallergenic derivative of Bet v 1 (BM4) was generated by grafting an epitope from apple Mal d 1 onto the corresponding position of the Bet v 1 backbone. The molecule was cloned, expressed in *E. coli* and purified to homogeneity. Circular dichroism and Fourier transformed infrared spectroscopy verified that the incorporation of a Mal d 1 epitope into the Bet v 1 backbone destabilized its structure leading to a protein unable to adopt the typical Bet v 1-like fold. The rearrangements lead to a significantly larger hydrodynamic radius of BM4; still the protein does not show increased tendency for oligomerization or aggregation. The changed overall fold of BM4 drastically reduced its IgE binding properties as demonstrated by direct and indirect ELISA as well as basophil mediator release assays. In contrast, activation of human peripheral blood mononuclear cells with the mutant allergen was elevated when compared to wild type Bet v 1. In animal models this strong immunogenicity of BM4 led to the induction of high titers of blocking antibodies cross-reactive with wild type Bet v 1. The elevated immunogenicity is based on a more efficient uptake of BM4 by dendritic cells and an enhanced proteolytic processing of the molecule by endo-/lysosomal proteases which could be demonstrated by *in vitro* experiments. Excellent production yields, good stability, reduced allergenicity and retained T cell activating properties turn BM4 into the ideal candidate for a safe and effective immunotherapy of birch pollen allergic individuals. We propose, that epitope grafting within the identified region presents a method, generally applicable for altering IgE binding properties of Bet v 1 family members from pollen as well as food sources. This work was supported by Biomay, the Christian Doppler Research Association, the University of Salzburg and Land Salzburg.

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High-throughput NMR authentication of food allergens

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For *in vitro* allergy diagnosis, purified food allergens have to meet high standard quality criteria. 1D 1H-NMR analysis can play a significant role to assess the conformation and to contribute to the authentication of allergens. Furthermore, this method allows to i) perform a structural comparison of allergens within a protein family, ii) identify the thermal stability of food allergens and iii) detect structural differences between recombinant and natural allergens.

Methods: The most important food allergens from cow's milk (Bos d 4, 5, 8) , hen's egg (Gal d 1-5), fish (Gad m 1), shrimp (Pen a 1), peanut (Ara h 1-3), hazelnut (Cor a 1-4, Cor a 8,9,11), celery (Api g 1,4,5) and apple (Mal d 1-4) and peach (Pru p 1,3) were purified according to established purification protocols and their allergenic activity tested.

Primary and secondary structures were verified by N-terminal sequencing, MALDI-TOF-MS analysis and far-UV CD-

spectroscopy. IgE binding capacity was tested in ELISA and immunoblotting assays using allergic patients' sera.

The presence and extent of the tertiary structure was assessed by two 1D-1H-NMR experiments, using a 700MHz field at 298K and under variable buffer conditions.

If available the consistency of the allergen's spectra was compared with structural information already available.

Thermal stability of purified nsLTPs from different sources was investigated and NMR spectra compared. NMR spectra of recombinant Cor a 8 was compared to the natural counterpart as well as for the fish parvalbumin. For Bet v 1 related food allergens their NMR spectra were compared regarding relevant structural differences.

Results and conclusions: The analysis of the 1D-1H-NMR spectra allowed the classification of the allergens into molecules whose spectra showed the unquestionable features of a rigid and extended tertiary structure, molecules without a rigid tertiary structure and allergens which displayed both features, tertiary structure with flexible and mobile regions. Furthermore, differences regarding thermal stability within a protein family were detected based on NMR spectra. In summary, 1D-1H-NMR proved a highly useful method requiring low protein concentrations, without 15N and 13C labeling for the structural authentication of allergens even when there is a limited quantity of protein available.

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Hymenoptera venomics: A systematic approach to solve current limitations in diagnosis and treatment of insect venom allergy

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Hymenoptera venoms are a major cause of IgE-mediated anaphylaxis. Surprisingly, they have remained inadequately characterised with regard to their full allergen composition. Although extract-based diagnosis and venom immunotherapy (VIT) are generally accepted procedures, limitations still exist, such as treatment failures and systemic side effects under VIT, sIgE double-positivity and CCD-reactivity, and lack of sensitivity of diagnostic tests. Thus, there is considerable interest in improving diagnostic tests and VIT in both, honeybee (HBV) and vespid venom (VV) allergy through identification of new allergens in a systematic approach.

A full set of *A. mellifera* (Api m 1-3/5-10) or *V. vulgaris* (Ves v 1-5) allergens was either produced in *E. coli* or as glycosylated protein in two insect lines (*T. ni*; *S. frugiperda*) with varying degrees of fucosylation (CCD). Melittin (Api m 4) was used as synthetic peptide. sIgE reactivity was analysed with sera from venom-allergic patients. All allergens were also applied in CD63 basophil tests. For several allergens (e.g., Api m 1/3/5/10; Ves v 3/5) human monoclonal IgE antibodies (mAb) were generated.

Expression in insect cells provided venom allergens all capable of activating patient basophils, thus indicating a native-like structure. This was further supported by conserved enzymatic activity of recombinant insect cell-derived Ves v 1 (PLA1), Ves v 2a/Api m 2 (HYA), and Ves v 3/Api m 5 (DPPIV). Insect cell-based expression appears highly suitable to analyse the impact of glycosylation on sIgE reactivity. Sf9- in contrast to *T. ni*-derived venom allergens lack α-1,3-core fucosylation (CCD), thus enabling differentiation of sensitisation with clinical impact from mere CCD-based cross-reactivity. The venom allergen panel together with cognate IgE mAbs is an ideal tool to investigate the relevance of low abundance allergens. Although recognized by patient sIgE in >50%, it was surprising to find that Api m 3 and Api m 10 are – despite their presence in crude HBV – apparently absent or vastly underrepresented in VIT preparations.

Our data suggest that the differential recombinant expression and application of a full panel of hymenoptera venom allergens provides superior solutions to currently existing limitations in diagnosis and therapy of HBV and VV allergic patients.

ORAL ABSTRACT SESSION 12: Genetic and Clinical Aspects of Inflammatory Disorders

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Molecular genetics of eczema and atopy

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Atopic eczema (AE) (atopic dermatitis, eczema) is one of the most common chronic skin disorders and a major manifestation of atopic disease. It has been firmly established that eczema has a strong genetic component. However, the understanding of its complex genetic susceptibility remains poor compared to other multifactorial diseases. Mutations in the gene encoding the epidermal barrier protein filaggrin (*FLG*) have been shown in numerous studies and meta-analyses to clearly convey a strong and consistent risk for AE. Recently, association studies have been revolutionized through new high-throughput SNP genotyping platforms and knowledge gained from the HapMap project. Thus, it has now become easier to perform genome-wide association studies (GWAS) to identify common low-risk variants. We recently performed the first only genome-wide screen for eczema, in which we identified a robust site of association on chromosome 11q13.5 in an intergenic region between the two annotated genes CIIorf30/EMSY and LRRC32/GARP. This association has recently been confirmed in another independent population of Irish pediatric eczema cases. The same study detected an additional susceptibility SNP located within the *HRNR* gene, which encodes the filaggrin-related protein hornerin, as well as again confirmed the association of four prevalent *FLG* null mutations with eczema in a European population, underlining the importance of inherited abnormalities of epithelial structural proteins in the pathogenesis of AE and related traits. Fine mapping and functional studies are ongoing to identify the causative variant(s) at the 11q locus. Genome-wide association studies have also identified novel susceptibility genes for asthma (*ORMDL3, CHI3L1, PDE4D*) and total IgE (*FCER1A*, *RAD50*). These genes have been replicated and functional studies have supported their relevance in asthma and atopy. A second international large-scale GWAS for eczema is currently underway. Although GWAS present many logistical, technical and biostatistical challenges, and have several limitations,

they clearly represent a powerful tool for the genetic dissection of complex diseases. Our results demonstrate that if such studies are performed under stringent conditions, are sufficiently powered, and are thoroughly reproduced, they have the potential to significantly advance our understanding of the pathogenetic mechanisms of atopic diseases.

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***In silico* identification of hair root tissue-specific gene expression profiles of atopic dermatitis**

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Rationale: We have reported disease-specific mRNA expression profiles of hair root tissue of skin diseases such as atopic dermatitis (AD). In the present study, we have compared our microarray data of hair root to those of skin and bronchial epithelium derived from allergic diseases and controls.

Methods: We used Subio Platform (http://www.subio.jp/products/), software for omics data management. Five hairs with their roots were obtained from both affected and unaffected skin lesions on the head of 8 patients with atopic dermatitis (AD), 8 patients with psoriasis vulgaris and 3 healthy individuals. Total RNA was extracted from the hair roots and gene expression profiles were determined by GeneChip system. Microarray data of bronchial brushing samples of asthmatics and controls (GSE4302 dataset) and those of skin biopsy samples of AD and controls (GSE5667 dataset) were obtained from GEO database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gds).

Results: Among three chemokine transcripts (*CCL2*, *CCL17* and *CCL18*) markedly up-regulated in AD skin, only *CCL2* was detectably up-regulated in AD lesional hair root. A STAT6-sensitive skin barrier protein, loricrin gene was markedly down-regulated in both AD samples. Langerhans cell signatures such as *CD207* and *CD1a*, and *FCER1A* were markedly up-regulated in AD hair root but not in AD skin. Expression of mast cell tryptase genes were markedly up-regulated in bronchial brushing samples of some asthmatics and were detected at high levels in all skin samples. However, such transcripts specific to mast cells as well as those to basophils were almost under detection levels in hair root samples.

Conclusion: Examination of mRNA expression profiles in hair root tissue may provide important information about the activation status of cells in epidermis, including keratinocytes, hair follicular cells and langerhans cells but not mast cells. It may be a useful method to investigate local pathophysiology of skin diseases with minimal invasiveness.

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Tissue oriented approach to eosinophilic esophagitis

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Even though eosinophilic esophagitis (EoE) is defined histologically by the presence of >15 eosinophils per high power field within the epithelial layer of the esophagus, it is a very heterogeneous disease with respect to clinical presentation and response to therapy. We hypothesized that the immune mechanisms that lead to eosinophilic infiltration of the esophagus may also vary from patient to patient, which may correlate with response to a specific treatment.

To answer this question, esophageal biopsy samples from 20 patients (ages 3-16 years) with EoE were evaluated. Patients' records were reviewed for food allergy history, total serum IgE levels and treatment. Immunofluorescence studies and confocal microscopy were performed on esophageal biopsy samples using two different antibody sets. Primary antibodies against IgE, tryptase and IL-5 were used to test whether IL-5 positive mast cells expressed IgE, whereas eotaxin-3, IL-13 and IL-5 were used to evaluate the relationship between the major eosinophilic factors implicated in the mechanism of EoE.

Although, clinical phenotypes were uniform regarding multiple food allergies and elevated serum total IgE titers (349 IU/L +/- 102), the tissue phenotypes were heterogenous, with four different staining patterns detected in confocal microscopy. In half of the samples (n=10), there was co-localization of IgE, IL-5, and tryptase. In the remainder, either only IL5 co-localized with tyrptase (n=4), or did not (n=3), suggesting its non-mast cell origin. Finally, there was no significant staining either for IL-5 or IgE in 3 samples. Patients with biopsy samples where IL-5 and IgE co-localized to mast cells responded the best to food avoidance determined by resolution of the esophageal eosinophilia at repeat endoscopy. Patients with pathologies revealing either a non-mast cell source of IL-5 or no IL-5 staining, did not improve by food avoidance and required swallowed steroids for clinical remission.

These data, for the first time, provide evidence that there is immunological heterogeneity of EoE at the tissue level. This different set of markers could not only provide a diagnostic tool, but also be utilized for clinical decision making to choose between different treatment options.

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Budesonide as induction treatment for active eosinophilic esophagitis in adolescents and adults: A randomized, double-blind, placebo-controlled study

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Background Eosinophilic Esophagitis (EoE) is a clinico-pathological defined condition characterized by PPI-refractory esophagus-related symptoms in combination with a dense esophageal eosinophilia. Topical corticosteroids have shown to be an efficient therapy in children and, on an anecdotal base, in adults. The purpose of this study is to assess the efficacy of budesonide in adolescents and adults with active EoE and to analyze the reversibility of symptoms and signs in EoE.

Methods In this randomized, double-blind trial, 36 adolescent or adult patients (m/f = 31/5; mean age 35.7yrs, range 17-65) with active EoE (>20 eos/hpf and dysphagia) swallowed either nebulized aqueous budesonide suspension formulation at a dose of 1 mg twice daily or placebo twice daily for 15 days. Pre- and post-treatment the activity of the disease was assessed clinically, endoscopically, histologically and via biomarkers in the blood and in the tissue. Symptoms were recorded with a diary. The primary endpoint was histological reaction, defined as remission (max <5eos/hpf), response (max 5-20eos/hpf) or persistent active inflammation (max >20eos/hpf).

Results At baseline, both groups were histologically comparable (p=0.52, Wilcoxon test). After the 15-day induction therapy, the remission rates were 61.1% (11/18 patients) in the budesonide

group as compared with 5.7% (1/18 patients) in the placebo group (p=0.0009). The rates of response were 11.1% (2/18 patients) in the budesonide group and 5.7% (1/18 patients) in the placebo group. The inflammation was still active in 27.8% (5/18 patients) of the budesonide group and in 88.9% (16/18 patients) in the placebo group. The treatment was well tolerated and no serious adverse events occurred. Among the endoscopical sings, white exudates and red furrows disappeared in parallel with the eosinophilic infiltration, whereas corrugated and solitary rings persisted.

Conclusions A 15-day course with the topical corticosteroid budesonide is highly effective in inducing a remission in adolescent and adult patients with active EoE. Symptoms correspond fairly well with degree of inflammation. White exudates and red furrows are associated with active inflammation and are reversible, whereas corrugated and solitary rings do not respond to anti-inflammatory therapy and likely reflect fibrosis. However, the long-term management of this chronic-inflammatory disease remains further investigation.

ORAL ABSTRACT SESSION 13: Clincial Aspects of Allergic Disorders

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Inhaled interferonß in the treatment of exacerbations of asthma, COPD and H1N1 variant influenza

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In asthma we and others have previously shown that common respiratory virus infection accounts for up to 85% and 65% of exacerbations in asthma and COPD respectively. Using experimental virus infection of volunteers and brushed airway epithelial cells in monolayer culture or differentiated at an air-liquid interface, we have further shown that in asthma common cold viruses, such as the major and minor classes of rhinovirus (RV), preferentially infect the airway epithelium as a consequence of defective TLR3-induced production of IFNβ by airway epithelial cells in association with increased production of the Th2 cytokine, thymic stromal lymphopoietin. We now report that airway epithelial cells of young children and from the airways of lifelong smokers are also highly susceptible to RV cell cytotoxicity. In all cases exogenous IFNβ resulted in a profound (70-90%) inhibition of cell cytotoxicity and viral shedding. Similar results have also been obtained with the variant H1N1 influenza in A459 alveolar-like cells. By showing impaired IRF7 induction with DS RNA (polyIC) by asthmatic epithelial cells, but normal downstream antiviral responses to exogenous IFNβ, the "lesion" appears to be in the proximal rather than the distal anti-viral response. We have developed a formulation of IFNβ1a suitable for administering as an aerosol. In a series of ascending dose safety studies in normal and asthmatic volunteers both single and repeated inhaled IFNβ1a for up to 2 weeks resulted in no local or systemic side effects. During these safety studies anti-viral biomarkers were measured in serum and induced sputum. In sputum, IFNβ1α administration stimulated a 2.04-fold increase in neopterin levels (equivalent to that seen in the circulation during treatment of MS patients with systemic IFNβ) compared to only a 1.07-fold rise in circulating neopterin. Inhaled IFNβ in asthmatics also produced a 4-95-fold increase in sputum cell mRNA encoding the anti-viral proteins 2',5'oligoadenylate synthetase (OAS1b), myxovirus resistance protein 1 (Mx1) and chemokine (CXC motif) ligand 10 (IP10) that persisted for 24 hrs post-dosing, but was lost by 3 days. These results confirm the safety and provide proof of mechanism for daily inhaled IFNβ1α for preventing virus-induced exacerbations and possibly swine influenza.

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Distinct mechanisms for bronchoprotective and anti-inflammatory effects of endogenous prostaglandin E_2

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Prostaglandin (PG) E_2 is considered a pro-inflammatory mediator because non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenases (COX) that catalyze biosynthesis of PGs. In subjects with asthma, inhalation of PGE_2 however causes bronchodilation and inhibition of induced bronchoconstriction.

We have characterised the receptors and iso-enzymes involved in the action of PGE_2 using guinea-pig trachea (GPT) airway smooth muscle (ASM), and mice ovalbumin (OVA) models of airway hyperresponsiveness (AHR) and allergic inflammation.

Using new in-house developed primers in ASM of GPT, qRT-PCR documented that expression of $EP_1 > EP_2 > EP_3 >> EP_4$, and COX-1 > COX-2. Tissue bath studies showed that EP_1 mediated constriction and EP_2 relaxation in GPT, whereas EP_3 and EP_4 had no effect on ASM.

In both an eosinophil-driven short protocol and a longer protocol implicated to be mast cell-dependent, the non-selective COX inhibitors diclofenac and indomethacin enhanced OVA-elicited AHR to methacholine (MCh), measured by flexiVent methodology in BALB/c mice, whereas the OVA-induced increase of inflammatory cells in bronchioalveolar lavage fluid (BALF) was inhibited by both COX-inhibitors. The selective COX-1 inhibitor FR 122,047 only enhanced AHR whereas the selective COX-2 inhibitor lumiracoxib mimicked the inhibitory effect of the unselective NSAIDs on BALF cells.

Finally, the OVA-induced increase in AHR was greater in C57 BL6 microsomal PGE_2 synthase (mPGES) knock-out (KO) mice than in wild type. The levels of PGE_2 in BALF were reduced by about 50% in the KO mice (580 ± 208 [n=10] vs 314 ± 179 [n=9] pg/mL;$p<0.05$) whereas the cellular response to OVA challenge was unchanged. Addition of diclofenac to KO mice further reduced PGE_2 in BALF (113 ± 36 [n=10], $p<0.05$ vs KO only), and increased AHR to MCh above the response in KO mice alone.

This is the first report on the expression of receptors and enzymes in the PGE_2 pathway in GPT, and the first study of the effect of mPGES KO in a mouse model of allergic inflammation. The findings support that the EP_2 receptor is a promising target for bronchoprotection, whereas inhibition of mPGES may carry the side effect to increase AHR.

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Rash oral immunotherapy for the treatment of hen's egg- and cow's milk-induced anaphylaxis

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Rationale: Recently rush oral immunotherapy (ROIT) is reported to be effective for the treatment of persistent hen's egg (HE) or cow's milk (CM) allergy.

Methods: We explained methods and effectiveness of ROIT to guardians of 94 patients aged 6 years or older with anaphylactic type of HE or CM allergy. After obtaining informed consent with the approval of ethical committee, oral food challenge tests were performed in 67 children to confirm the application of the ROIT. DBPCFC was done in 31 patients (12 HE and 19 CM) with positive systemic reactions to determine the thresholds. During the ROIT, patients were encouraged to eat 1/16-1/4 HE- or CM-dried powder of the thresholds in admission, and were asked to take those a few times a day. The doses were doubled each time and ROIT was performed over 6-9 days in the presence of oral anti-histamine drug. After the ROIT, patients were asked to take their maximum dose of HE or CM everyday to maintain the effect. Patients visit outpatient clinic once a month to confirm the level of their thresholds.

Results: Thresholds of heated-HE was 10.4±5.8g (mean ± SD), and that of CM 8.1±6.7ml. In the presence of oral anti-histamine, threshold values were increased to 18.4±10.1g of heated HE and 29.8±17.1ml of CM. During ROIT period, 11 patients (91.7%) out of 12 HE allergy could eat a heated-whole egg without symptom. Seventeen patients (89.5%) of 19 CM allergy could drink 200ml of CM. Mechanisms of ROIT seemed to be suppression of Ag –induced SPT followed by suppression of Ag-specific IgE Ab. The size of erythema of SPT was suppressed to 62.8% (HE, n=12) and 58.1% (CM, n=19) at 1 month after the treatment. Interestingly, basophil histamine release was not inhibited by ROIT.

Conclusion: In our study ROIT was effective in about 90% of cases. ROIT can mostly prevent HE- or CM-induced anaphylaxis. The one mechanism seems to be the suppression of IgE-mediated mast cell activation but not basophil activation. We can not conclude yet whether or not these patients would develop real tolerance or just maintain the symptom-free condition by the therapy.

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The World Allergy Organization survey on global availability of essentials for the assessment and management of anaphylaxis

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Background: The availability of medications, supplies and equipment for the assessment and management of anaphylaxis by physicians in healthcare settings worldwide is unknown.

Objective: To ascertain the global availability of these essentials.

Methods: A survey instrument was developed and sent by e-mail to a non-randomized sample of allergy/immunology specialists in the World Allergy Organization in 2008. Responses were tabulated by country.

Results: Physicians from 44/52 different countries on six continents completed the survey, for an 85% response rate. Anaphylaxis guidelines were in use in 70% of the 44 countries. Laboratory tests to confirm the clinical diagnosis of anaphylaxis were available in 37% of the countries. Medications for the management of anaphylaxis were available as follows: epinephrine (adrenaline) in ampules for injection, 100% of the countries; any intravenous glucocorticoid, 89%; beta-2 adrenergic agonist by nebulizer/compressor, 86%; any intravenous H_1-antihistamine, 77%; any intravenous H_2-antihistamine, 70%; glucagon, 73%; atropine, 73%; dopamine, 86%; noradrenaline, 70%; and vasopressin, 64%. All the basic medications considered to be necessary were available in 54% of the countries. Supplies and equipment for anaphylaxis treatment were available as follows: for giving supplemental oxygen, 95% of the countries; for intubation, 89%; for giving intravenous fluid resuscitation, 91%; for monitoring oxygenation using pulse oximetry, 91%; and for continuous non-invasive cardiac monitoring, 81%.

Conclusions: Essential medications, supplies and equipment for the assessment and management of anaphylaxis by physicians in healthcare settings were not universally available worldwide in 2008. Residents and travellers who experience anaphylaxis in many of the world's countries might be at increased risk of fatality because of lack of availability of these essentials. The World Allergy Organization Anaphylaxis Guidelines that are in development should list the basic medications, supplies and equipment that are absolutely necessary, as well as those that are optimal.

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Allergies have a socioeconomic impact: A model calculation and what allergologists can do to save costs

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Allergies pose an increasing problem to all industrialised and industrializing countries, with at least 30% of the younger population being affected. However, available data show that only 10 % of the patients are being managed appropriately. The majority of the allergic population are younger than 65, which means a majority are working or at school or university. The major threat of allergies is that they, if not correctly managed, reduce mental capacity. This leads to an impairment of cognitive functions and can affect long-term memory and intellectual capacity. Table I shows the minimum and maximum impact of allergies in a minimum and maximum assumption for one person.

	Average loss of efficient work per year / employee (in h)	Total amount of costs per year (30,00 € per working hour)
Minimum: 60 days of symptoms, 10% reduction in efficiency	**34,30**	**1029,00**
Maximum: 120 days of symptoms, 30% reduction in efficiency	**205,80**	**6174,00**

Table I: The calculation is based on the following assumptions:

- 5 working days / 40 hours work per week
- 30,00 € per hour according to Eurostatistics, including all overhead
- allergic symptoms 60 – 120 days (if patient sensitized to tree and grass pollen 180 days, dust mite 365 days)
- only 10 % of patients are being treated correctly
- reduction of cognitive functions by 10% - 30% (available data show 30% reduction)

If these individual values are calculated for the whole work time of the EU the following results are seen.

	Employees in Europe suffering from airway allergy (20-35%)	90 % are not being treated correctly	Average. loss of work per year / employee (in h)	Total amount of loss of work per year (in h)	Total amount of costs per year (30,00 € per working hour)
Minimum: 60 days of symptoms, 10% reduction in efficiency	**$39,6*10^6$**	**$35,6*10^6$**	**34,30**	**$1.2*10^9$**	**$36.7*10^9$**
Maximum: 120 days of symptoms, 30% reduction in efficiency	**$69,3*10^6$**	**$62.4*10^6$**	**205,80**	**$12.8*10^9$**	**$385.1*10^9$**

Table II: Example for employees in Europe: 198 million employees, ca. 20-35% suffering from allergic airway diseases of 2005 (Eurostatistic)

The calculation is based on the following assumptions:

- 5 working days / 40 hours work per week
- 30,00 € per hour according to Eurostatistics, including all overhead
- allergic symptoms 60 – 120 days (if patient sensitized to tree and grass pollen 180 days, dust mite 365 days)
- only 10 % of patients are being treated correctly
- reduction of cognitive functions by 10% - 30% (available data show 30% reduction)

However, even worse is the impact on pupils and students in school and university. The socioeconomic influence of allergies could be reduced if modern management was consistently.

In summary, these calculations provide preliminary data on the cost of impaired work and school efficiency by allergies. In the European Union, allergy-associated reduced work-efficiency leads to an avoidable economic damage most likely exceeding 100 billion Euros a year.

POSTER SESSION 5: Clinical Aspects of Allergic Disorders

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Human B regulatory cells. Do they really exist?

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B cells do not only produce antibodies, but memory B cell which express certain cytokines can suppress antigen specific T cell responses. A crucial regulatory function of B cells has been demonstrated in experimental autoimmune encephalitis, chronic colitis, contact hypersensitivity collagen-induced arthritis and non-obese diabetic mouse models. IL-10 secretion in murine B cells appears to be restricted to a distinct subset of $CD1d^{hi}CD5^{+}$ B cells. A regulatory B cell subset remains to be elucidated in humans. We hypothesize that if a B cell plays an anti-inflammatory role, the antibody isotype produced by this B cell after differentiation to a plasma cell will be anti-inflammatory. The aim of this study is to identify and characterize a human IL-10-producing B cell subset and to determine whether these cells differentiate into IgG4-secreting plasma cells. Treatment with a TLR9 agonist induced strong B cell proliferation and high levels of IL-10 production in purified peripheral as well as tonsil-derived B cells. Furthermore, such treatment led to the production of IgG4 at the mRNA as well as the protein level and ligation showed that these cells produce higher levels of IgG4 than cells that do not secrete IL-10. Furthermore, IL-10-producing B cells show strong suppressive capacity on antigen-specific T cell proliferation whereas B cells that did not produce IL-10 were unable to suppress such a response. These data demonstrate that a suppressive memory B cell subset exists in human which produces mainly IgG4 after differentiating into a plasma cell.

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Nasal polyp polyclonal IgE is functional in response to allergen and SEB

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Introduction: Evidence is accumulating that Staphylococcus aureus plays an important role as disease modifier in upper and lower airway disease, esp. severe nasal polyps and asthma, by stimulating T-cells via classical enterotoxins (SAEs). Those superantigens also can induce local formation of polyclonal IgE-antibodies, the role of which is unknown.

Methods: Nasal tissue and serum was obtained from 26 adult patients (12 allergic rhinitis patients and 14 nasal polyp subjects), in whom SPTs were also performed. Total IgE and specific IgE-abs to inhalant allergens and enterotoxins were determined in both, serum and tissue. Tissue fragments were stimulated with either TCM, 10 µg/ml ε-chain specific anti-human IgE antibody, 10 µM ionomycin, 0.5 µg/mL SEB or grass and house dust mite allergens for 30 minutes. PGD2 was measured to demonstrate mast cell degranulation.

Results: In allergic rhinitis patients, reactivity of tissue mast cells upon allergen exposure and presence of specific IgE-abs to inhalant allergens corresponded in 23/24 cases for tissue IgE-abs and in 21/24 cases for serum IgE-abs. In allergic rhinitis patients, total IgE in serum and inferior turbinate tissue homogenates highly correlated ($r=0.91$, $p<0.0001$).

In contrast, in nasal polyp patients, reactivity of tissue mast cells upon allergen exposure and presence of specific IgE-abs to inhalant allergens or SEB corresponded for tissue, but not for serum IgE-abs. In SAE-IgE positive polyp tissue, we were able to degranulate tissue mast cells with allergens to which these patients did not show any serum specific IgE or SPT positivity. Total IgE was at least double as high in tissue compared to serum in these patients; total IgE in serum and tissue did fail to show a meaningful correlation.

Conclusions: We here for the first time demonstrate that SAE-induced local IgE antibodies in nasal polyp tissue are functional and able to activate mast cells upon allergen challenge; specific IgE antibodies in nasal polyp tissue can be found independently of their presence in serum. We postulate that superantigen-induced polyclonal IgE-abs in airway disease contribute to chronic inflammation by continuously activating mast cells.

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Reduced expression of claudin-1 in the epidermis of atopic dermatitis subjects disrupts barrier function and enhances susceptibility to HSV-1 infections

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Rationale: Barrier disruption is a cardinal feature of atopic dermatitis (AD). There are two barrier structures found in the epidermis the stratum corneum (SC) and immediately below this are the tight junctions (TJ). TJs control the diffusion of fluid, electrolytes, macromolecules and pathogens. We evaluated the expression/function of the TJ protein, claudin-1 in epithelium from AD and nonatopic (NA) subjects and screened two North Americans populations for SNPs in *CLDN1*.

Methods: Expression profiles of nonlesional epithelium from extrinsic AD and NA subjects were generated using Illumina's HumanRef-8 BeadChips. Claudin-1 expression was evaluated by tissue staining and qPCR. We screened 27 haplotype-tagging SNPs in *CLDN1* in two independent groups of AD and NA (European American, EA; n=404 and African American, AA; n=323). The role that claudin-1 expression plays in TJ function and HSV-1 infectivity was assessed using a knockdown approach (siRNA) in undifferentiated and differentiated primary human foreskin keratinocytes (PHFK).

Results: Claudin-1 was one of only two TJ proteins that was reduced in AD nonlesional skin, which we confirmed at both the mRNA and protein levels. In both AA and EA populations, individual *CLDN1* SNPs were associated with AD, AD severity and an AD subphenotype recognized for their predilection for widespread cutaneous herpes simplex virus (HSV)-1 infections ($p<0.05$). *In vitro*, we noted claudin-1 protein expression and membrane localization in Ca^{+2}-differentiated PHFK coincident with an increase in transepithelial electrical resistance (TEER) and reduced permeability. Claudin-1 knockdown significantly reduced TEER (49±3.2%; $p<0.035$), increased sodium fluorescein permeability (2.3±0.5 fold) and enhanced HSV-1 infectivity.

Conclusions: Claudin-1 is a crucial component of the epidermal barrier and is a novel susceptibility gene for AD.

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Asthma prediction in school children: The value of combined IgE-antibodies and obstructive airways disease severity score

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Background: Allergic sensitization increases the risk for asthma development. In this prospective birth cohort (Environment and Childhood Asthma) study we hypothesised that combining quantitative measures of IgE antibodies (∑-IgE) and Severity score of obstructive airways disease (OAD) at two years (Severity score) of age is superior to predict current asthma (CA) at 10 years than either measure alone. Secondarily, we assessed if gender modified the prediction of CA.

Methods: A follow-up study at 10 years of age was performed in 371 two year old children with recurrent (n=219) or no (n=152) bronchial obstruction with available serum analysed for ∑-IgE to common food and inhalant allergens through a panel test, Phadiatop Infant ® (Phadia, Uppsala, Sweden). Clinical variables included allergic sensitisation and exercise testing to characterise children with CA vs not CA at 10 years and the Severity score (0-12, 0 indicating no OAD) was used to assess risk modification.

Results: Severity score alone explained 24 % (Nagelkerke $R^2 = 0.24$) of the variation in CA whereas ∑-IgE explained only 6% ($R^2 = 0.06$). Combining the two increased the explanatory capacity to $R^2 = 0.30$. Gender interacted significantly with ∑-IgE; whereas Severity score predicted CA in both genders, the predictive capacity of ∑-IgE for CA at 10 years was significant in boys only.

Conclusion: Combining ∑-IgE to inhalant allergens and Severity score at two years was superior to predict asthma at 10 years than either alone. Severity score predicted CA in both genders, whereas ∑-IgE significantly predicted CA in boys only.

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Rupatadine improves nasal symptoms, airflow and inflammation in patients with persistent allergic rhinitis: A pilot study

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Background: Nasal obstruction is the main symptom in patients with allergic rhinitis and may be measured by rhinomanometry. Rupatadine is a new antihistamine with potential antiallergic activities. The aim of this pilot study was to evaluate nasal symptoms, nasal airflow, and nasal mediators in patients with persistent allergic rhinitis, before and after treatment with rupatadine.

Materials and Methods: Twenty patients with persistent allergic rhinitis were evaluated, 15 males and 5 females (mean age 35 ± 9.1 years). All of them received rupatadine (10 mg/daily) for 3 weeks. Nasal and ocular symptoms (measured by VAS), rhinomanometry, and nasal mediators (ECP and tryptase) were assessed in all subjects before and after treatment.

Results: Rupatadine treatment induced significant symptom relief (both nasal and ocular, respectively $p=0.005$ and $p=0.0004$), including obstruction ($p=0.0015$) and significant increase of nasal airflow ($p=0.0025$). Moreover, there was a significant difference of nasal mediators.

Conclusions: This pilot study demonstrates the effectiveness of rupatadine treatment in: i) improving nasal and ocular symptoms, ii) increasing nasal airflow, iii) exerting antiallergic activity in patients with persistent allergic rhinitis. These results support the previous effectiveness of rupatadine in the treatment of persistent allergic rhinitis.

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Resistin-like molecule-β promotes human airway fibroblast cellular proliferation and differentiation and may be involved in airway fibrosis

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Resistin-like molecule-beta (RELM-β) is a member of the cysteine-rich cytokine family members of which are involved in insulin resistance and inflammation. RELM-β is expressed *in vitro* in human airway epithelial cells, fibroblasts and smooth muscle cells. RELM-β was shown to enhance airways inflammation and remodelling in a mouse model. However, its biological role the in human airway is uncertain. We sought to examine whether RELM-β enhances airway fibroblast cell proliferation, differentiation and ECM protein expression and to examine the possible mechanisms involved. The effects of recombinant RELM-β on proliferation and differentiation of a human lung fibroblast line (MRC-5) and primary cultured airway fibroblast cells were evaluated by MTT assay and Western blotting, respectively. Effects of RELM-β on expression of TGF-β1, TGF-β2 and ECM proteins were measured using ELISA, RT-PCR, qPCR and Western blotting. RELM-β significantly increased MRC-5 fibroblast cellular proliferation and expression of α- smooth muscle actin, and also enhanced expression of TGF-β1, TGF-β2, collagen I and fibronectin at both the mRNA and protein levels. Similar data were obtained from primary cultured human airway fibroblast cells. Our data suggest that RELM-β may be involved in airways fibrosis and remodelling by inducing proliferation of airways fibroblast cells and promoting their differentiation into myofibroblasts, as well as by increasing expression of ECM proteins including collagen I and fibronectin. These latter effects may in turn reflect RELM-β-induced TGF-β1 and/or TGF-β2 expression.

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Increased release of cysteinyl-leukotrienes in ex vivo stimulated sputum cells from subjects with aspirin-intolerant asthma

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Aspirin intolerant asthma (AIA) is a syndrome characterized by hypersensitivity to all Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), asthma and chronic nasal afflictions including polyposis and rhinosinusitis. It is known that subjects with AIA release increased amounts of cysteinyl-leukotrienes (CysLT) when challenged with NSAIDs *in vivo*. However, there is no *in vitro* test to diagnose AIA. The study therefore tested the hypothesis that sputum cells from subjects with AIA could be activated *ex vivo* by challenge with lysine-aspirin in the test tube.

Sputum induction was performed at baseline and two hours after a lysine-aspirin bronchoprovocation in ten subjects with AIA and nine subjects with aspirin-tolerant asthma (ATA). Two million living sputum cells were isolated and incubated for 15 min at 37 °C with lysine aspirin (100 μM), calcium ionophore A23187 (2.5 μM) or solvents (saline or ethanol). CysLTs levels in the supernatants were analysed by enzyme immunoassay.

Aspirin did not release CysLTs in sputum cells from patients with AIA, whereas the ionophore challenge caused significantly increased CysLT release in isolated sputum cells from both AIA and ATA groups. The levels were however significantly higher in the AIA group both at baseline (AIA vs ATA, 3.43 ng/million cells vs 0.54 ng/million cells, $p<0.05$) and when the cells were isolated after the lysine-aspirin bronchoprovocation (3.90 ng/million cells vs 0.89 ng/million cells, $p<0.01$). There was a significant correlation between sputum eosinophil count (%) and increased CysLT release caused by ionophore ($r=0.58$ at baseline and $r=0.61$ at post-provovation, both $p<0.01$).

Ex vivo stimulation of isolated sputum cells provides a new method to standardize measurements of cellular capacity for CysLT production. Sputum cells from subjects with AIA released greater amounts of CysLTs than those from subjects with ATA. NSAID intolerance could however not be triggered in the test tube by exposure of isolated sputum cells to lysine aspirin.

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Psychosomatic aspects in allergy: Organically unexplained symptoms in allergy inpatients

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Background: Allergic diseases may cause a relevant psychological burden due to severe and sometimes life-threatening symptoms. Other patients presenting with suspected allergies eventually suffer from allergologically unexplained symptoms.

Methods: We studied psychobehavioral characteristics in 204 (73% female, mean age 43±13y) patients of our allergy unit hospitalized for challenge tests. Initially, patients were interviewed by a psychiatrist and completed self-report questionnaires. After work-up, the degree of organic explicability was rated by allergists. Blind double ratings were performed to assess interrater reliability in every 6th patient. Group comparisons were conducted between patients with and without organic symptom explicability, and - to control for effects of the workup situation – with 49 patients hospitalized for specific hymenoptera venom immunotherapy (VIT).

Results: In 27.4% of tested patients, symptoms were not or almost not organically explained. Patients without organic symptom explanation showed a higher rate of psychosomatic disorders (45.5% vs. 20%, $p<0.001$). The best predictors for a lack of organic explicability were “dissatisfaction with medical care” (AUC 0.65; 95%CI 0.56-0.73; $p=0.001$), a “disturbed interactional behavior” (AUC 0.67; 95%CI 0.59-0.76; $p<0.001$) and a negative countertransference (AUC 0.73; 95% CI 0.65-0.81; $p<0.001$). Compared to VIT patients, food and drug work-up patients were characterized by a higher level of psychological distress, specific psychobehavioural characteristics, and a higher psychiatric comorbidity.

Conclusions: The psychological burden of a supposed allergy should be recognized independent of symptom etiology in all patients in allergy diagnosis strategies. Interdisciplinary screening for specific mental disorders presenting with physical symptoms is helpful. A difficult patient-doctor interaction is a possible predictor of organically unexplained symptoms.

Abstracts

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Evidence of receptor revision and class switching to IgE in nasal polyps

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Background: Chronic rhinosinusitis with nasal polyps (NP) is often characterized by local polyclonal hyper-immunoglobulinemia E and specific IgE to Staphylococcus aureus enterotoxins (SAE). We aimed to investigate if IgE is localy produced, if this IgE is functional and if this process is linked to RAG expression (receptor revision).

Methods: In nasal tissue homogenates of patients with NP (n=12), allergic rhinitis (AR, n=8) and normal controls (NC,n=8) we measured concentrations of total IgE and IgE to SAE by ImmunoCAP. By quantitative PCR we determined IL-21, BAFF, activation-induced cytidine deaminase (AID), germline gene transcripts (GLT) for IgE. Paraformaldhyde fixed sections from nasal mucosa were immunostained for the coexpression of RAG1 and RAG2 in T-cells (CD3), B-cells (CD20) and plasma cells (CD138) using dual immunofluorescence(IF). To test if the tissue IgE is functional, RBL (rat basophilic leukaemia) cells were sensitized with NP homogenates containing IgE and stimulated with grass pollen extracts. Cell degranulation was detected by measuring beta-hexosamidase release.

Results: NP-samples show significant increased levels of IgE, BAFF, IL-21, AID, epsilon GLT and mature epsilon mRNA. Here we provide evidence that germinal center reactions occur in these structures. We show that up to 30% of B cells, plasma cells and T cells in nasal polyps re-express the recombinantion-activating genes, RAG1 and RAG2, required for local receptor revision. Tissue IgE levels correlated significantly to $RAG1^+$ and $RAG2^+$ in NP-tissue. We show that *S. aureus* enterotoxin-specific IgE, detected in 7/21 polyps, is associated with higher levels of RAG1 and RAG2 expression.

Finally, grass pollen extracts were able to degranulate RBL cell loaded with IgE from NP homogenates from atopic and non-atopic NP-patients. Thus this local IgE is functional.

Conclusion: Thus, our results strongly suggest that *S. aureus* enterotoxins induce an inflammatory response sufficient to induce germinal center reactions, receptor revision and class switch recombination, resulting in diversification of the local B cell repertoire and the synthesis of functional polyclonal IgE.

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Expression of CD30+ and CD45RO+ in acute atopic dermatitis

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We investigated the presence of CD 30+, CD 45RO+, CD4+ and CD3+ cells in the inflammatory infiltrate of acute phase of atopic dermatitis (AD) (compared with that of allergic contact dermatitis). Skin biopsies were obtained from 12 patients suffering from acute (AD) and from 13 with acute allergic contact (nickel induced) dermatitis as a control.

All patients fulfilled the diagnostic criteria of Hanifin and Rajka, had positive family history of atopy, and showed elevated serum IgE level, positive skin prick test and no infectious episodes in preceding month. None of the patients had been on antibiotics, steroids, antihistamines, or immunosuppressive drugs. The control subjects had no history of atopy and showed normal serum IgE level. CD 30 expression was evaluated by immunohistochemistry –Streptavidin-Biotin-Avidin method with semiquantative evaluation in lesional skin biopsies. High CD 30 expression was observed in a remarkable proportion of infiltrating cells.

The analyses of CD 30+, CD45RO+, CD4+ and CD3+ cells in dermis and in epidermis showed a much wider range of values and statistically higher median ($p<0.01$) in the inflammatory infiltrate of acute (AD) compared with that of allergic dermatitis. Our results showed an association between CD30 expression and AD, but not in allergic contact dermatitis. CD 30 expression in (AD) might be helpful in histological differentiation of these disorders and in further characterization of atopy patch testing.

Abundant CD45RO+ cells were detected both in (AD) and in allergic contact dermatitis lesions. The results suggest a specific regulatory function of CD 30+ T cells in acute dermatitis.

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Clarithromycin reduces disease burden after an acute asthma exacerbation. An open randomized trial

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Therapy for asthma exacerbations has remained unchanged for many years. Macrolides are not currently included among the recommended treatments for either acute or chronic asthma, although the initial experience with macrolides in treating asthma dates back more than 40 years. Macrolides can modulate neutrophil migration, the oxidative burst in phagocytes, the production of proinflammatory mediators (e.g., TNF-a, IL-8, IL-6, and IL-1b), and eosinophilic inflammation.

We have conducted a randomized open study on the effects of clarithromycin, as additional therapy in school-age children with acute asthma exacerbations. The primary outcome was days without symptoms during 12 weeks, after receiving or not clarithromycin 15mg/kg daily for 3 weeks. The presence of atypical bacteria was evaluated with antibodies and PCR. Two hundred children were screened; forty presented with an exacerbation during a predefined 2 year period. Cases and controls were well matched in respect to baseline characteristics (demographic, disease severity, previous use of medication). Children who received clarithromycin had more symptoms-free days comparing with the control group [78(2) vs 69(6) days respectively, $p<0.00001$]. Furthermore, the duration of the exacerbation was shorter in the clarithromycin group [5 (1) vs 7.5(1) days, $p<0.00001$], as well as the duration and number of subsequent episodes [0.5(3) vs 7(5) days, $p<0.00001$ and 9 vs 19 algorithm-defined exacerbations respectively, $p=0.013$). Only 2 children, one from each group, had evidence of atypical bacterial infection.

Clarithromycin is effective in reducing disease burden after an acute asthma exacerbation in children. The role and potential of macrolides in the treatment of asthma exacerbations should be assessed extensively.

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Treatment of severe asthma and food IgE-mediated diseases with anti-immunoglobulin E antibody

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IgE plays a central role m mediating allergic responses. Omalizumab, a recombinant humanized monoclonal antibody, is used in the treatment of patients with severe IgE-mediated asthma. In Italy, Omalizumab is only approved for treatment of patients with severe persistent asthma, not controlled by following drugs: oral and/ or inhaled corticosteroids, long and/or short-acting beta-agonists, cromolyn/nedocromil, leukotriene modifiers, methylxanthines and anti-cholinergics.

A female patient (23 years old) experienced several severe attacks of IgE-mediated asthma and food allergy to milk (anaphylaxis). This patient was admitted to emergency room several times (in last year; 2 times for anaphylaxis to milk and 3 for attacks of severe asthma). Before treatment with Omalizumab, she had an asthma symptom score of 7 [scale from 0 (least) to 9 (severe)]. The total IgE concentrations were 306 IU/mL; specific IgE against *Dermatophagoides Pteronyissinus* (91 KU/L), *Dermatophagoides Farinae* (79 KU/L), and to milk allergenes: alfa-lactalbumin (16 KU/L), beta-lactoglobulin (14 KU/L), casein (19 KU/L). The patient was treated with Xolair® every four week 0.016 mg/kg/IU (IgE/mL). After 16 weeks of treatment with Xotair®, total IgE were 220 lU/ml. No accesses to intensive care unit was needed during the treatment and the asthma symptom score decreased to 3. The patient tolerated a diet including cow's milk and its derivatives.

Omalizumab was helpful in the treatment of this patient with severe asthma and severe episodes of milk-induced anaphylaxis.

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Environmental contact allergens in adult atopic dermatitis

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Background: There is an increasing number of adult atopic dermatitis (AD) with a 1-9%. word-wide prevalence. Environmental contact allergens (ECA) have been rarely studied in adult AD population.

Aim of the study was to investigate the hypersensitivity rate of adult AD patients to common ECA and evaluate these data in intrinsic and extrinsic AD patients.

Methods: 65 AD patients (SCORAD 2.9-12.3) over 18 years of age were studied. 50/65 have been identified with an extrinsic type AD, while 15/65 as to have an intrinsic type. Intrinsic AD was diagnosed in patients without allergic rhinitis or conjunctivitis, without bronchial asthma, with normal serum IgE level, no specific serum IgE and without Prick test positivity. Epicutaneous environmental contact serie (*Brial-Allergen D-Greven Panel)* were performed in every patient according to the current international methodics.

Results: ECA hypersensitivity was present in 49% of the AD patients (women 51%, men 44%). Common allergens were nickel (20%), thiomersal (10.7%) and mercury chlorid (6%). We observed contact hypersensitivity in 5 patients in the intrinsic group: 40% of the intrinsic women and 20% of the men were sensitised. 50 AD patients were selected in the extrinsic group, in ECA series late positivity was seen in 27 patients (54%). Most common allergens were nickel, mercury compounds and lanalcolum.

Conclusions: The sensitivity rate of ECA is remarkable in adult AD. Women and extrinsic AD patients are sensitized to ECA in higher rate. The most common ECA by adult AD patients are nickel, mercury compounds, in extrinsic AD lanolin as well.

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Immunoglobulin free light chains in rhinitis

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Background: Rhinitis is characterized by allergen-induced mast cell activation. Although increased levels of allergen-specific IgE correlate with rhinitic symptoms in many cases, also a substantial number of patients do not show local or systemic increases in total or specific IgE levels. In the latter group other mechanisms may be involved in triggering allergen-induced mast cell activation and rhinitic symptoms. In previous work, we have shown that immunoglobulin free light chains can elicit antigen-specific mast cell activation. In this study, we investigated the involvement of FLC in allergic and non-allergic rhinitis.

Methods: Levels of tryptase, ECP, IgE and FLC in nasal secretions from allergic and non-allergic rhinitis patients and normal subjects were measured using specific ELISA's and UniCap technology. Immunohistochemistry and laser microdissection was used to analyze FLC expression on different cell types in nasal mucosa. Local FLC, IgE, tryptase and IL-5, 6, 8, GMCSF, IFN-g and TNF-a were measured at different time points after intranasal allergen provocation.

Results: Tryptase and ECP levels were highly increased in both allergic and non-allergic rhinitis when compared to control subjects. IgE levels were only significantly increased in allergic patients. FLC levels were increased in nasal secretions from non-allergic and allergic rhinitics. FLC-positive cells, both mast cells and plasma cells, were increased in allergic and non-allergic rhinitis patients compared to controls. Local allergen provocation of allergic subjects induced rapid secretion of tryptase, FLC, IgE, ECP and cytokines.

Conclusions: Local levels of FLC and FLC positive cell numbers in nasal mucosa are significantly increased in allergic and non-allergic rhinitis patients. Since mast cells were found positive for FLC, this supports a possible involvement of FLC in allergic and non-allergic rhinitis.

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Active transport of Bet v 1 allergen through nasal epithelium

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We used a top-down approach with a wide repertoire of wet lab and *in silico* techniques for analysing the pathogenesis of early events within the type I allergic reactions.

We identified a group of birch allergic subjects and verified their clinical symptoms during birch pollen season with positive skin prick

test. An age-matched group of healthy controls, with no symptoms and a negative skin prick test was also identified. We then collected conjunctival and nasal epithelial cell swabs and biopsies from all subjects during winter, when there was no birch pollen in the air and thus even the allergic patients did not present any symptoms. During this previously non-symptomatic period we could also apply *in vivo* perturbation by an application of pollen allergen to the conjunctiva as well as under the inferior meatus of the nasal cavity, which caused rapid symptoms in allergic subjects, but no manifestation in healthy controls.

Comparing the conjunctival epithelial cells and tissues from healthy and allergic subjects we show with light and electron microscopic analyses that the Bet v 1 birch pollen allergen bound to epithelial cells within minutes even during the non-symptomatic winter season only in allergic, but not in healthy individuals. It was also transported through the morphologically intact epithelium and reached mast cells located close to or under the basal lamina during the non-symptomatic winter season only in allergic patients. Co-localization together with caveolar markers and affinity experiments with mass spectrometry suggested that this traffic was dependent on caveolae. Finally, transcriptomics provided evidence that the respiratory epithelium differs between healthy controls and allergic patients already during non-symptomatic winter season and furthermore the unique sets of genes alter in both groups in response to the Bet v 1 birch pollen challenge.

Thus taken together, the application of discovery driven methodologies on human conjunctival and nasal epithelial cells and tissues can provide new hypotheses worth further analyses to the molecular mechanisms of a complex multifactorial disease such as the type-I birch pollen allergy. Learning novel, unpredictable aspects from complex immunological diseases can provide future targets for drug development.

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TGFß receptor expression is reduced in the airways in mild asthma compared to non-asthmatic controls

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Background: TGFβ is an activator of fibrosis and is suggested to act in airways remodelling in asthma. However, this cytokine is also an important mediator of suppression by regulatory T cells. We recently showed that TGFβ, activin and BMP signalling are activated upon allergen challenge in mild asthma and that these factors may act to reduce inflammation and initiate repair in the airway[1-3]. However activin and BMP receptors were deficient in mild asthma at baseline. Here we examined $TGF\beta_1$ ligand, receptor expression and signalling in mild asthma and associated the findings to a marker of remodelling.

Methods: Bronchial biopsies from 15 mild atopic asthmatics and six non asthmatic controls were examined by immunohistochemistry or *in situ* hybidization for expression of HSP47 (a marker of fibroblast collagen synthesis), $TGF\beta_1$ protein and mRNA, pSmad2 (activated signalling component for TGFβ), and the TGF receptors ALK 1, ALK5, and TβRII.

Findings: Numbers of HSP47+ fibroblasts were increased in the airways of asthmatics compared to controls suggesting active collagen synthesis even at basal conditions ($p<0.03$). Cells expressing TGFβ protein and mRNA did not differ between asthmatics and controls, nor was there a difference in numbers of cells expressing pSmad2. ALK1 ($p<0.001$) and ALK5 ($p<0.005$) were both reduced in expression in airway epithelium from asthmatics, and ALK1 staining cells were reduced in the submucosa whilst ALK5+ cells were very scarce in the submucosa in asthma or control biopsies. TβRII staining did not differ between asthmatics and controls.

Conclusions: These data argue against a role for TGFβ in the active airway remodelling detected even in baseline asymptomatic asthmatics. Together with our previous findings these data suggest that the asthmatic airway may be deficient in the regulatory activity of the TGF family (TGFβ, activin and BMPs) to control inflammation but that these factors may be activated as a response to airway injury, reducing inflammation and initiating repair.

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Topical lysine aspirin in aspirin exacerbated respiratory disease

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Introduction: Aspirin sensitivity, present in 10-20 % of adult asthmatics ,often with polypoid rhinosinusitis considerably affects quality of life. Oral aspirin desensitization ameliorates symptoms, reduces exacerbations and hospitalization (1) but high doses (over 600mg) risk adverse events(1); low dose (100mg) is ineffective(2).

Topical nasal lysine aspirin alone (LAS) reduces cysteinyl leukotriene 1 receptors(3) and improves the nasal airway when used in addition to intranasal steroids in aspirin sensitive(4), but not aspirin tolerant patients.

We describe an outpatient procedure combining lysine aspirin nasal challenge with subsequent self- administered updosing nasally, and later orally in positive patients. The final dose equates to 100mg aspirin, 60mg administered nasally.

Results: 208 (109 male, 99 female, mean age 47.2 ± 13.7) challenges were performed between 1995 and 2008 with one adverse reaction: facial angioedema without respiratory compromise (incidence 0.4%).

Sixty four percent reacted to nasal challenge, 13 % to subsequent oral challenge and 23% were negative.

Of 76 patients who agreed to use nasal LAS, 66 remained on treatment at 3 months and 22 at 12 months. Reasons for stopping were compliance, nasal or gastrointestinal discomfort , worsening symptoms.

LAS therapy was associated with significant improvements in nasal inspiratory peak flow (pre 143+-1.6, 3 months 159.7+-1.5($p<0.02$), 12 months 174.3+- 4,($p<0.03$) and eNO: 14.8+-0.57pre and 8+- 0.7 at 12 months,($P<0.02$).

Of the 22 patients on 12 months LAS therapy none needed emergency or hospital asthma treatment, 4 had oral prednisolone.

In 20 patients who discontinued LAS 6 had extra GP visits for asthma**,** 5 attended A&E, 6 were hospitalized with asthma exacerbations and 13 had prednisolone courses.

Conclusion: LAS challenge is safe; 100mg aspirin is effective when given partly nasally.

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Food allergy related quality of life in patients with birch associated food allergy

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Background: Pollen associated food allergy (FA) is noticed in about one quarter of patients with birch pollinosis (Osterballe 2009). We aimed at investigating the impact of food allergy on quality of life (QoL) in our patients with birch associated food allergy.

Methods: 69 consecutive patients (48 f, 21 m, median age 43 years) with type-1 allergy to birch (pos. skin prick test and/ or specific IgE >CAP1) were investigated by a standardized questionnaire for (i) foods having elicited immediate type reactions at consumption, (ii) for type of clinical reactions (oral allergy syndrome/OAS, OAS and generalized symptoms [OAS+GS] at skin, lung, GI-tract, cardiovascular system, generalized symptoms [GS] only) and (ii) by Food Allergy QoL Questionnaire (FAQLQ; Flokstra de Blok 2009) applying a scale of 0 – 6 (not/extremely affected). It comprises 35 questions on allergen avoidance and dietary restrictions (AADR), emotional Impact (EI), risk of accidental exposure (RAE), FA related health (FAH), FA independent measures (FAIM). A scale of 0 – 6 (not/extremely affected) was recoded as 1-7. Patients were grouped as follows: A: 1-5 eliciting foods, B: 6-10 foods, C: > 10 foods.

Results: Eliciting foods and type of reactions (calculated according to reported consumption): apple 82% (OAS 66% / OAS+GS 27% / GS 1%), hazel 75% (54/31/15%), soy drink 60% (21/35 patients with previous consumption; 38/38/18%), carrot 51% (54/29/17%), peach 38% (61/27/12%), cherry 49% (82/12/5%), walnut 48% (61/23/16%), kiwi 40% (55/30/15%) etc. FAQLQ gave the following values (group A with n= 26 / B n=21 / C n=22): AA: 2.2 / 3.3 / 4.2, EI: 3 / 4.4 / 4.6, RAE: 2.8 / 4.6. / 4.8, FAH: 5 / 5 / 5.8, FAIM 3 / 3 / 3.7 Comparison of patients having ever reacted to soy (n=25) with those who exclusively had reacted to any other birch associated food (n=44) gave the following values: AA: 3.6 vs. 3, EI: 4.6 / 3.5 RAE: 4.1 / 3.5, FAH: 5.7 / 5 , FAIM 3.3 / 3.

Conclusion: Birch associated food allergy in our patients was most frequently noticed at consumption of apple, hazel and soy drinks. OAS was the most prominent clinical reaction but a relevant number of patients also reported generalized symptoms. Quality of life was mainly affected regarding FAH (worries about health) and emotional impact (i.e. fright, apprehensibility at consumption). Patients with reactions to soy showed high emotional impact values. All values of FAQOQ deteriorated with number of foods that had elicited symptoms.

POSTER SESSION 6: Treatment of Immune Disorders

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Hypoallergenic derivatives of the major birch pollen allergen Bet v 1 obtained by rational sequence reassembly

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Birch pollen belongs to the most common causes of allergic symptoms affecting an estimated 100 million of people worldwide. We have constructed by rationale molecular reassembly three recombinant hypoallergenic derivatives of the major birch pollen allergen, Bet v 1 for immunotherapy. They were generated by re-assembly of codon-optimized genes coding for Bet v 1 fragments containing the elements for the induction of allergen-specific blocking IgG antibodies and the major T cell epitopes. The proteins were expressed in Escherichia coli and compared with the Bet v 1 wild-type protein by chemical and structural methods, regarding IgE- and IgG-binding capacity, in basophil activation assays and tested for the in vivo induction of IgG responses which block patients' IgE recognition of Bet v 1. All three mosaic molecules showed strongly reduced IgE reactivity and allergenic activity. Immunization with the recombinant hypoallergens induced IgG antibodies which inhibited allergic patients' IgE reactivity to Bet v 1 stronger than those induced with the rBet v 1 wildtype allergen. We report the generation and preclinical characterization of three hypoallergenic rBet v 1 derivatives with suitable properties for immunotherapy of birch pollen allergy.

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***Phleum pretense* depigmented allergoids contain the same allergen profile as native extracts**

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RATIONALE: Chemically modified allergen vaccines are widely used in Europe and their clinical efficacy has been demonstrated in numerous studies. Although different studies have been published in the last year, the characterization and standardization of these extracts remain challenging. The objectives of this study were to

determine whether allergens present in native extracts are detectable in depigmented-polymerized extracts and to demonstrate the immunogenic capacity of depigmented-polymerized allergen vaccines.

METHODS: Native and depigmented-polymerized allergen extracts of *Phleum pratense* (Laboratorios LETI S.L.) were manufactured and the major allergen content (group 5) measured in the last step where the allergens can be detected. Polymerized samples were resuspended and digested with trypsin or pepsin. Resulting peptides were separated by reversed phase capillary HPLC coupled to a mass spectrometer (Q-Tof) and sequenced. Based on an ELISA sandwich with monoclonal antibodies, the presence of the group 5 was also demonstrated. 2-D immunoblot analyses were performed to determine the immunogenicity in rabbits.

RESULTS: Native extract contained 42.1µg/mg Phl p5, whilst depigmented extract contained 54.8µg/mg. Sequences of Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, P hl p 7, Phl p 11 and Phl p 12 were identified in the native extracts and their corresponding depigmented-polymerized extracts and the presence of Phl p 5 was confirmed by sandwich ELISA. Immunization of rabbits with a depigmented-polymerized *Phleum pratense* extract containing 70µg/ml Phl p 5, induced specific IgG against allergens and isoforms present in native extracts.

CONCLUSIONS: These findings suggest that modified (depigmented-polymerized) extracts of *Phleum pratense* have a high probability of containing the allergens present in native extracts and can induce specific IgG against individual allergens and isoforms.

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Protection of the respiratory tract by an oral killed non-typeable haemophilus influenzae vaccine

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Recent studies emphasise the critical role played by T cells derived from Peyer's patches in the protection of airways from pathogens, independent of IgA antibody. We have completed a double blind placebo controlled trial of an orally administered inactivated non-typeable *Haemophilus influenzae* (NTHi) product (HI-164OV) in 38 subjects with severe COPD (FEV_1 of less than 50% predicted normal) over a winter period, to assess benefit in terms of frequency and severity of acute exacerbations. The results are summarised:

	Treatment Group		
	HI-164OV (18)	**Placebo (20)**	**% Protection (p-value)**
Number of acute episodes (per subject)			
• Total	1.22	1.45	16 (0.55)
• Antibiotic-treated	0.83	1.15	28 (0.33)
• Corticosteroid-treated	0.28	0.75	63 (0.05)
Proportion of subjects with episodes:			
• Total	0.72	0.65	0 (NS)
• Corticosteroid-treated	0.22	0.55	56 (0.07)
Antibiotic Treatment (per subject)			
• courses	1.06	2.4	56 (0.03)
• total days	7.56	27.15	72 (0.01)
• ≥ 3 antibiotic courses	0.11	0.55	80 (0.01)
Duration of episodes mean days (range)	14.3 (3-81)	22.7 (4-74)	37 (0.01)
Admission to Hospital (Respiratory Event)	1	11	90 (0.04)

In addition, there was a reduction in number of pathogens isolated from sputum ($p<0.05$), and there was no stimulation of mucosal IgA antibody. A specific T cell response is induced by HI-164OV ($p=0.05$) as is a reduction in secreted lysozyme (as a biomarker of mucosal inflammation) ($p<0.05$). It is concluded that oral immunisation can induce T cell-dependent protection of the airways with a reduction of pathogen load, reduced luminal inflammation and reduced frequency and severity of acute exacerbations.

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Allergen-specific IgG antibodies for treatment and prevention of type I allergic reactions

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Several studies report that allergen-specific IgG antibodies induced through allergen-specific immunotherapy contribute to reduction of allergic reactions as well as to prevention of allergic sensitization when transferred from mother to child.

Our aim was to investigate the impact of passive administration of allergen-specific IgG antibodies on allergic disease and on allergic sensitization in a mouse model of grass pollen allergy. For this purpose, BALB/c mice were first sensitized to the major grass pollen Phl p 5 and then divided into two groups. Group 1 received with Phl p 5–specific polyclonal rabbit IgG antibodies whereas group 2 was injected polyclonal IgG specific for Bet v 1, an unrelated pollen allergen. Blood samples taken before and after IgG antibody administration were compared regarding their IgE reactivity to rPhl p 5 in ELISA and their immediate allergic reactions using rat basophil leukemia (RBL) assays. It revealed that passive application of Phl p 5-specific IgG reduced the IgE binding to Phl p 5 up to 80% while Bet v 1-specific IgG had no effect on the Phl p 5-specific IgE level. Moreover, Phl p 5-induced mediator release was inhibited up to 90% after passive administration of Phl p 5-specific IgG. To investigate the effect of prophylactic administration of allergen-specific IgG antibodies on allergic sensitization, naïve BALB/c mice received Phl p 5-specific polyclonal rabbit IgG and for control purposes, polyclonal rabbit IgG specific for an unrelated grass pollen allergen, Phl p 2. Mice were then sensitized to Phl p 5. Blood samples were taken before prophylactic antibody treatment and after sensitization and analyzed regarding their IgE and IgG1 reactivity to Phl p 5 in ELISA and their immediate allergic reactions in RBL assays and intra-dermal skin tests. Prophylactic administration of Phl p 5-specific IgG prevented the development of Phl p 5-specific IgE and IgG1 and hence no basophil degranulation and skin reactivity was elicited by Phl p 5.

Passive administered allergen-specific IgG antibodies are able to abrogate established manifestations of allergic disease and when applied prophylactically to prevent allergic sensitization.

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Peptides derived from the major grass pollen allergen Phl p 5 lacking IgE reactivity and by-passing T cell recognition induce allergen-specific protective IgG responses

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Allergen-specific immunotherapy is the only allergen-specific and disease-modifying approach for the treatment of allergy but IgE-mediated and T cell-dependent side effects limit its broad applicability. The major timothy grass pollen allergen Phl p 5a contains the majority of group 5 specific IgE epitopes and is involved in more than 80% of allergic reactions in grass-pollen allergic subjects. We synthesized seven Phl p 5-derived peptides comprising each 31-38 amino acids, which according to IgE epitope mapping data and secondary structure predictions, are located within or close to IgE binding sites. None of the peptides showed IgE-binding or allergenic activity as demonstrated by IgE-binding- and basophil activation assays in grass pollen allergic patients. The peptides were further studied regarding the induction of T cell proliferation and secretion of pro-inflammatory cytokines in allergic patients. Furthermore, they were coupled to the carrier protein KLH (keyhole limpet hemocyanin) to select peptides which induce allergen-specific IgG antibodies which cross-react with group 5 allergens from other grasses and inhibit allergic patients IgE binding to the allergen as well as allergen-induced basophil activation. In these experiments we could identify three non-allergenic peptides which induced allergen-specific protective IgG responses and additionally showed a strongly reduced allergen-specific T cell activation and cytokine secretion as compared to Phl p 5.These peptides may now be used for the formulation of a carrier-bound peptide vaccine with a reduced ability to cause allergen-specific IgE- and T cell-mediated side effects.

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Generation of Cetuximab-like canine IgE and IgG antibodies for comparative oncology

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Background: Whereas the IgG class is clinically applied as passive immunotherapy of cancer patients, no data on the efficacy or risk of IgE anti-cancer antibodies are available yet. IgE antibodies cooperate with distinguished cytotoxic and phagocytic effector cells with demonstrated tumoricidic properties. As the canine species has i) a similar expression pattern of IgE receptors on the same cell types as humans, and ii) develop tumors showing a similar antigen profile as human patients, dogs represent relevant models for allergooncology.

Objective: The present study aimed to validate whether canine epidermal growth factor receptor (EGFR) is a relevant immunotherapeutical target for dogs and, to construct canine anti-EGFR IgE and IgG anti-EGFR antibodies for side-by-side comparison.

Methods and Results: Due to a 91% identity between human and canine EGFR, the anti-human EGFR antibody Cetuximab recognized canine EGFR homologue on several canine cancer cells in FACS. To construct canine Cetuximab.like IgE and IgG, we amplified DNA encoding for the heavy chain constant region of immunoglobulin epsilon from normal canine PBMC genomic DNA. Immunoglobulin gamma heavy chain and immunoglobulin Kappa constant regions were amplified from canine PBMC cDNA. The PCR products were cloned into a eukaryotic expression vector for Cetuximab, pBud-225huIgG1. The 1.6 Kb IgE and 1.1 Kb IgG heavy chain constant region PCR products, the pBud-225huIgG1 vector and the 300bp Ig kappa constant region PCR product were cloned into EF-1α or CMV multiple cloning site, respectively. By realtime PCR, a number of cDNA clones encoding Cetuximab-like canine-mouse chimeric IgE and IgG (subclasses A, B, C and D) were constructed. The cDNA sequence analysis showed the clones are consistent with the published data of canine IgE.

Conclusion: Also in canine cancer EGFR is a relevant tumor target. The newly generated canine anti-EGFR antibodies will allow comparing the efficacy of IgE and IgG classes side by side in vitro, in vivo and in veterinarian clinical oncology. It is expected that future comparative oncology studies will provide the basis for application of recombinant anti-tumor IgE antibodies as passive immunotherapy of human patients with cancer.

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A mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides and a probiotic strain suppresses the allergic sensitization against whey protein in mice

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Cow's milk allergy (CMA) is the most common food allergy in children. So far, no effective treatment is available to prevent or cure food allergy.

Aim: The purpose of this study was to examine whether dietary supplementation with a specific prebiotic mixture (Immunofortis®) and/or a specific probiotic strain, *Bifidobacterium breve M-16V*, could reduce allergic sensitization in a murine model of orally induced IgE-mediated CMA.

Methods: Three-weeks-old female C3H/HeOuJ mice were fed diets containing 2% Immunofortis® and/or the *B. breve M-16V*. Mice were orally sensitized with whey for five times during weekly intervals. The acute allergic skin response was determined by measuring ear swelling. Antigen-induced anaphylaxis was scored. Furthermore whey-specific serum immunoglobulins and mouse mast cell protease (mMCP-1) were determined.

Results: Immunofortis® 67.4 ± 7.2 µm, p<0.05), *B. breve M-16V* (83.1 ± 8.1 µm, p<0.01) and the combination (29.9 ± 6.4 µm, p<0.01) effectively suppressed the allergic skin response as compared to whey sensitized mice fed control diet (117.6 ± 8.6 µm). The anaphylactic reaction was less severe in mice fed the probiotic diets (p<0.01). The whey-specific IgE and IgG_1 responses were not affected, however IgG_{2a} was increased in Immunofortis® (2332 ± 776 AU, p<0.05), *B. breve M-16V* (3209 ± 11104 AU, p<0.01) and synbiotic (3047 ± 621 AU, p<0.01) fed animals. MMCP-1 concentrations, reflecting mucosal mast cell degranulation, were reduced by the synbiotic diet (4.0 ± 0.9 vs 17 ± 4.5 ng/mL).

Conclusion: Dietary supplementation with Immunofortis® and *B. breve M-16V* reduces the clinical allergic response in a murine model of IgE-mediated hypersensitivity that closely mimics the human situation. This model shows the potential for dietary intervention with pro- pre and synbiotics in reducing the allergic response. Recent data indicate that in a human proof of concept clinical trial the same effects were measurable.

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A peanut allergoid with reduced IgE-binding and maintained immunogenicity

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Background: Peanuts are one of the most common foods responsible for food-induced anaphylaxis. A curative treatment is not yet available for peanut-allergic patients. A preparation of Ara h2 and Ara h6, the most potent natural peanut allergens, for immunotherapy is being developed.

Methods: Ara h2 and Ara h6 are purified by extraction from de-fatted peanut powder using liquid chromatography. The preparation is chemically modified by reduction of the intramolecular disulfide bonds of Ara h2 and Ara h6, and alkylation of the resulting free sulfhydryl groups in some examples followed by modification with glutaraldehyde. The resulting allergoids were adsorbed to aluminium hydroxide. The allergen-specific IgE binding potential was measured using an inhibition ELISA and IgE-immunoblotting. Short-term Ara h2/6-specific T cell lines (TCLs) generated from the PBMCs of 6 peanut-allergic patients were tested to determine the *in vitro* immunogenicity of the native and allergoid Ara h2/6 preparations. Proliferation was determined through measuring ^{3}H-thymidine incorporation. An *in vivo* immunogenicity model was set up in BALB/c mice to study the induction of IgG antibodies following their immunization with native and modified Ara h2/6 preparations.

Results: IgE-immunobloting showed a strong decrease of IgE binding for the allergoid preparations. The IgE inhibition ELISA showed that the binding to the Ara h2/6 allergoid preparations is reduced by > 99% as compared to that of the native Ara h2/6 preparation. The Ara h2/6 allergoid preparations are still capable to induce proliferation in TCLs of patients with clinically established peanut allergy, to a variable extent depending on the type of modification. Additionally, *in vivo* IgG responses in Balb/c mice immununized with the allergoid preparations are detected that are cross-reactive with the native Ara h2/h6 preparation.

Conclusion: *In vitro* and *in vivo* test models have shown that it is possible to prepare Ara h2/6 allergoids with a strongly reduced allergenicity compared to its native counterpart, but with immunogenicity that is retained to a large extent.

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A Bet v 1 peptide vaccine by-passing T cells and IgE

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Allergen-specific immunotherapy represents the only causative treatment of type I allergies, but its success is limited by the use of crude allergen extracts. Knowledge of the disease-eliciting molecules has lead to several clinical trials using purified recombinant allergens. It has become possible to selectively target the immune system using various allergen derivatives. Based on the dissection of B and T cell epitopes of the major birch pollen allergen Bet v 1 we produced a carrier-bound B-cell epitope containing peptide vaccine. The surface-exposed Bet v 1 peptides lack IgE reactivity and allergenic activity. Upon immunization they induced IgG antibodies which blocked allergic patients IgE-reactivity to Bet v 1. In this study we selected three of these Bet v 1-derived peptides comprising 20-30 amino acids, which were devoid of Bet v 1-specific T cell epitopes, coupled them to KLH and adsorbed the conjugate to aluminium hydroxide to obtain a Bet v 1-specific allergy vaccine. Groups of BALB/c mice where immunized with the peptide vaccine before or after sensitization to rBet v 1. Bet v 1-specific antibody titers were measured by ELISA, and T cell responses to Bet v 1, KLH, and the peptides were analyzed by spleen cell proliferation assays. Prophylactic as well as therapeutic peptide vaccination induced a robust Bet v 1-specific IgG_1 response without priming/boosting of Bet v 1-specific T cells. The peptide vaccine suppressed Bet v 1-specific T cell responses both, in a prophylactic and therapeutic model of Bet v 1 allergy. Furthermore the peptide vaccine induced no anaphylactic IgE antibody response, while the induced IgG antibodies were shown to inhibit IgE-mediated effector cell activation. The described strategy should be useful for the development of therapeutic and prophylactic peptide vaccines, which avoid IgE and T cell-mediated side effects.

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Alpha-MSH and fragments: Mediators of neuroinflammation with a therapeutic potential

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Neuropeptides are now well appreciated to exert cytokine like effects and to function as mediators of immunity and inflammation. Among these neuromediators α-melanocyte-stimulating hormone (α-MSH) derived from the proopiomelanocortin was found to exert potent immuno-regulatory and anti-inflammatory activities. The biologic activities of α-MSH are exerted via direct effects on cells of the immune system as well as indirectly via affecting the function of resident non-immune cells. Most of these effects are mediated via specific melanocortin receptors (MC-R) in particular MC-1R and MC-3R which are expressed on both immunocompetent as well as non-immune cells. α-MSH affects several pathways implicated in regulation of inflammatory responses such as NF-κB activation, expression of adhesion molecules and chemokine receptors, production of proinflammatory cytokines and chemokines. Thus α-MSH modulates the proliferation, activity, and migration of inflammatory cells as well as programmed cell death. Moreover, α-MSH prevents the maturation of dendritic cells (DC) and thereby triggers the

generation of a subset of regulatory T-cells. The anti-inflammatory and immuno-modulatory effects of α-MSH have been confirmed in several animal models of inflammation such as irritant and allergic contact dermatitis, cutaneous vasculitis, asthma, inflammatory bowel disease and rheumatoid arthritis. Most of the anti-inflammatory activities of α-MSH can be attributed to its C-terminal tripeptide KPV. K(D)PT, a derivative of KPV corresponding to the amino acid 193-195 of IL-1β, is currently emerging as another tripeptide with potent anti-inflammatory effects. The anti-inflammatory potential together with the favourable physiochemical properties most likely will allow these agents to be developed for the treatment of inflammatory skin, eye and bowel diseases, allergic asthma, and arthritis.

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Vaccination with carrier-bound non-allergenic Bet v 1-derived peptides induces allergen-specific IgG in a non-allergic individual

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The major birch pollen allergen, Bet v 1, is one of the major respiratory allergens in Northern and Middle Europe. We selected two Bet v 1-derived peptides, which lack IgE-mediated allergenic activity and most of the Bet v 1-specific T cell epitopes and coupled them to the carrier protein KLH (keyhole limpet hemocyanin). In order to test whether this vaccine may be useful for prophylactic vaccination, a non-allergic person was given subcutaneous injections of the aluminium hydroxide-adsorbed peptide vaccine. The vaccine induced Bet v 1-specific IgG antibodies without any allergic sensitization as monitored by skin prick testing with Bet v 1 and studying Bet v 1-induced basophil activation. According to the hapten carrier principle, the Bet v 1-specific IgG antibody production was primarily driven by a *de novo* induced carrier-specific T cell response. The Bet v 1-specific IgG antibodies induced by the peptide vaccine inhibited Bet v 1-induced basophil activation and lasted for approximately 4 months.

This is the first demonstration that allergen-derived peptides lacking both allergenic activity and the majority of allergen-specific T cell epitopes can induce protective allergen-specific IgG responses without sensitization in a non-allergic person. Peptide allergy vaccines based on the concept of by-passing IgE- and T cell recognition may represent safe therapeutic allergy vaccine which may also be considered for prophylactic vaccination

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Immunological changes associated with sublingual immunotherapy efficacy

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Background: Sublingual immunotherapy is now well established as a valid treatment for type I respiratory allergies, but immune mechanisms involved are still partially unknown. We have investigated this topic in the context of a randomised, double-blind placebo-controlled study conducted in an allergen challenge chamber.

Methods: We monitored changes in 140 immune parameters in a cohort of 82 grass pollen allergic patients receiving once daily a sublingual 300IR grass pollen tablet or a placebo for 4 months. Rhinoconjunctivitis symptoms were evaluated before, and after 1 week or 1, 2, 4 months of treatment following allergen challenge in a pollen chamber. Basophil activation, CD4+ T cell and antibody responses were assessed using a battery of phenotypic and functional assays performed in the blood, serum, nasal secretions and saliva.

Results: A significant clinical response was observed in the group receiving the active treatment, as early as after the first month and was maintained throughout the fourth month. An improvement versus placebo of 29.3% for the mean rhinocojunctivitis symptom score was observed at end point. Responders were defined individually as patients with at least a 50% decrease of their symptom score after SLIT. Comparing immune responses from patients belonging to each of the four groups, including Placebo responders / Placebo non responders / Active responders / Active non responders, we could establish that: (i) some Placebo responders exhibit strong grass-pollen specific antibody responses in nasal mucosa, (ii) No differences between Active responders and other groups of patients are observed with respect to CD4+ T cell responses (assessed in terms of Th1, Th2, Th17, or regulatory cytokine production, T Reg markers, T cell proliferation) or basophil activation in the blood, (iii) Active responders are heterogeneous, encompassing both "immunoreactive" patients with strong grass pollen-specific IgG, IgE and IgG4 responses (possibly blocking antibodies) induced during SLIT, as well as "non immunoreactive" responders with no detectable immune responses distinguishing them from patients receiving placebo.

Conclusions: Patients benefiting from SLIT are heterogeneous and other immune mechanisms distinct from T Reg induction and blocking antibodies are involved in SLIT efficacy.

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A cat vaccine based on carrier-bound Fel d 1-derived peptides for by-passing allergen-specific IgE and T cell reactivity

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Allergen-specific immunotherapy (SIT) is the only disease-modifying therapy for IgE-mediated allergy but can induce IgE- and T cell-dependent side effects. We have developed a vaccine for cat allergy based on a recombinant fusion protein consisting of the PreS domain of Hepatitis B Virus (HBV) and two surface-exposed peptides derived from the major cat allergen, Fel d 1. The peptides were selected from the Fel d 1 sequence

to eliminate allergen-specific IgE and T cell reactivity. Using a murine model, it is demonstrated that the vaccine induces allergen-specific IgG antibodies which inhibit allergic patients IgE reactivity to similar extent as IgG antibodies induced by vaccination with complete Fel d 1. According to the hapten-carrier principle described by Benacerraf the T cell help for the Fel d 1-specific IgG responses was derived from the HBV-carrier protein. Unlike the complete Fel d 1, the carrier-based vaccine did not induce reaginic Fel d 1-specific IgE antibodies indicating lack of allergenicity. The Fel d 1-specific IgG response could be augmented by pre-immunization with the HBV carrier protein and boosted without apparent need for T cell help by an isolated Fel d 1-derived peptide without HBV carrier. The vaccine can be easily produced by recombinant expression in *E. coli* in large quantities. It differs from the earlier described Fel d 1 T cell-epitope-based vaccines because it eliminates Fel d 1-derived T cell epitopes to a large extent. Furthermore, it lacks IgE-related allergenic activity but induces Fel d 1-specific protective IgG responses and thus should be useful for side-effect-free therapeutic and eventually prophylactic vaccination against cat allergy.

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Allergic responses in experimental autoimmune encephalomyelitis

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Allergic and autoimmune diseases have been considered to be at the opposite sides of the spectrum of the immune response. We have shown that in animal models of autoimmune disorders such as multiple sclerosis (MS) and insulin-dependent diabetes mellitus (IDDM), anaphylaxis can develop to 'self-peptides' that also are potential targets of autoimmune attack, findings that represent perhaps the most dramatic evidence of the occurrence of both allergic and autoimmune effector mechanisms in the same subjects. This observation prompted us to study the role of immune responses classically associated with allergy in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Understanding of how allergic responses can participate in EAE might help in the design of new strategies for the treatment for this disease in mice and, hopefully, in humans with MS, for which the currently available therapies are only partially effective.

EAE is a chronic inflammatory disease of the central nervous system (CNS) in which Th1 and Th17 cells autoreactive against myelin play a leading role. By using histamine deficient HDC -/- mice, we have already shown that EAE is more severe in mice with a profound histamine deficiency. By using the recently characterized model of mast cell-deficiency, c-*kit* mutant $Kit^{W\text{-}sh/W\text{-}sh}$ mice, we now show that EAE is exacerbated in mast cell deficiency, with increased myelin-specific T cell proliferation and pro-inflammatory cytokine production, and reduced IL-10 production. These findings are surprising considering that the findings contradict those reported from studies of EAE in the $Kit^{W/W\text{-}v}$ mast cell-deficient mouse model. $Kit^{W\text{-}sh/W\text{-}sh}$ mice engrafted i.v. with bone marrow-derived mast cells, which did not result in the appearance of mast cells in the CNS of the recipient mice, still developed exacerbated EAE. These results indicate either that protection from disease in wild type mice was exerted by mast cells mainly in the CNS and/or that exacerbation of disease in $Kit^{W\text{-}sh/W\text{-}sh}$ mice may reflect the contribution of cells other than mast cells that may be dysregulated in c $Kit^{W\text{-}sh/W\text{-}sh}$ mice (such as their increased numbers of neutrophils). Our findings also indicate the role of mast cells in the EAE model may be complex.

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ZaBeCor pharmaceuticals begins a phase II clinical trial with excellair in asthma patients

Schreiber AD

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Asthma is a chronic disorder in which the airways of the lungs become blocked or narrowed due to inflammation. It is estimated that asthma affects 300 million people worldwide. In the U.S. alone, asthma annually accounts for approximately 4,000 deaths, 500,000 hospitalizations, 1.9 million emergency room visits and 10 million physician visits. Thus, there is a pressing need for an effective, non-toxic treatment for asthma. To this end, we have developed Excellair, an siRNA administered with a hand-held nebulizer. Excellair is targeted specifically to Syk kinase, a critical initiating step in the intracellular signaling pathway for the release of many inflammatory mediators, including histamines, leukotrienes and cytokines.
Some of the current treatments for asthma and other inflammatory conditions, such as leukotriene inhibitors or TNF inhibitors, inhibit only one of the mediators of inflammation. By targeting Syk kinase, Excellair™ seeks to inhibit an initial signaling step of inflammation, thereby preventing the release of multiple inflammatory mediators. In a Phase I study, normal individuals and patients with mild to moderately severe asthma received 21 consecutive daily doses of Excellair. Excellair was well-tolerated by all patients (100%), with no serious adverse effects. In addition, 75% of patients with asthma who received Excellair™ reported improved ability to breathe freely or reduced use of their rescue inhaler. No patients on placebo reported improvement. These studies represent one of the first instances in which siRNA is successfully administered to the lung and one of the first to target asthma with siRNA. In addition to asthma, Excellair™ may have the potential to treat many different inflammatory diseases, including allergic rhinitis, rheumatoid arthritis, cardiovascular disease and other inflammatory disorders.

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Biologics targeting T cells and B cells improve atopic eczema

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Rationale: Atopic eczema (AE) is a chronic inflammatory skin disorder characterized by eczematous skin lesions, pruritus and typical histopathological features. Although T cells play a key role, B cells are also found in the dermal infiltrate. In 80% of AE patients, elevated total and specific IgE levels can be detected.

Aim: To evaluate the efficacy of alefacept, a LFA-3 (CD58) fusion protein, and rituximab, a monoclonal anti-CD20 antibody, in AE, two investigator-initiated, prospective, open-label pilot studies were conducted involving patients with moderate to severe AE.

Methods: Ten patients were treated with alefacept (15 mg, I.M., weekly, over 12 weeks). Six patients (2 males; mean age 39±7 years) with severe AE received two applications of rituximab, each 1000 mg IV two weeks apart. Peripheral blood cell analysis including immunophenotyping, clinical parameters (EASI, pruritus score, concomitant medication), skin histology, immunofluorescence analysis of skin infiltrating cells, cytokine expression by PBMC and by skin infiltrating cells on mRNA and protein levels were monitored.

Results: Upon alefacept therapy, all patients showed an improvement starting between 6 to 8 weeks after initiating therapy. The EASI significantly decreased (18.7±1.9 at week 0 versus 4.7±1.6 at week 12; $p<0.001$) paralleled by a reduction of pruritus. This effect lasted over the study period of 24 weeks (EASI 2.8±0.7). Upon rituximab, in all patients AE symptoms were significantly reduced. The EASI significantly decreased (before therapy: 29.4±4.3; week 8: 8.4±3.6; $p<0.001$). B cells were undetectable in the peripheral blood within 3 days. In contrast, IgE levels did not significantly change as a consequence of rituximab treatment. In both studies, histological alterations, such as spongiosis, acanthosis, and dermal infiltrate, dramatically improved. Inflammatory cell numbers in particular B and T cell significantly decreased in the skin. Skin cytokine expression, in particular of IL-13, also declined.

Conclusion: Treatment with both biologics, alefacept and rituximab, reduced skin inflammation in AE resulting in clinical improvement. They may present new therapeutic tools in patients refractory to topical treatment.

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A novel role of human polyclonal IgG in the maintenance of vascular function in humans

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Abnormalities in the immunological response and over-expression of adhesion molecules are key features of endothelial activation, which, in turn, induces endothelial dysfunction and atherosclerosis. However, it is unclear whether abnormalities in circulating antibodies directly contribute to the increased cardiovascular risk. Patients with common variable immunodeficiency (CVID), who are characterized by defective antibody production and increased circulating levels of adhesion molecules, might represent an ideal model to clarify whether circulating antibodies play a role in the maintenance of endothelial function. For this purpose, we studied thirty-five patients with CVID, eleven of them never treated (NT) with intravenous immunoglobulin (IVIG), and thirteen matched healthy controls (C). The twenty-four patients treated with IVIG (T-IVIG) had received the last IVIG five weeks before the baseline study. In all subjects, endothelial- (flow mediated dilation, FMD) and non-endothelial-mediated (NMD) vasodilation of the brachial artery were measured by ultrasonography technique. In all patients and C, FMD and NMD were measured at the basal state. In addition, the T-IVIG patients were studied one day, one, two, and three weeks after IVIG infusion (15 g). In NT and T-IVIG patients, FMD was similar (6.3±1.0% and 7.6±0.6, respectively, p=NS), but significantly impaired when compared to C (9.4±0.9%, $p<0.05$). NMD was similar in the three groups. In T-IVIG, FMD rose from baseline to 10.4±0.7% and 10.1±0.6 one day and one week after IVIG infusion ($p<0.001$ vs. baseline) and returned to baseline two and three weeks after IVIG infusion (8.0±0.5 and 7.4±0.6, respectively). NMD did not change after IVIG infusion. In conclusion: a) the defective antibody production in CVID is associated with endothelial dysfunction; b) IVIG infusion stimulates endothelial function of conduit arteries. These data support an antiatherogenic role of human polyclonal IgG.

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A universal concept for the generation of hypoallergenic fish vaccines

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Parvalbumin, a small calcium-binding protein, represents the major and sole allergen for 95% of patients suffering from IgE-mediated fish allergy. Owing to the high degree of cross-reactivity between parvalbumins from different fish species, fish allergic patients usually exhibit clinical symptoms after consumption of most fish species. However, some patients are able to tolerate certain fish species while being allergic to others. Such species-specific reactions point to the presence of species-specific allergen epitopes.

Specific immunotherapy, the only disease-modifying treatment of type I allergy, is not recommended for food allergies due to the risk of inducing severe anaphylactic reactions by systemic application of the allergens. By introducing four point mutants in the two calcium-binding regions of carp parvalbumin (Cyp c 1) we recently generated a hypoallergenic derivative, which represents a candidate molecule for treatment of fish allergy. We now raised the question whether exchanges of the corresponding, highly conserved amino acids in the calcium-binding sites of other fish parvalbumins, would also render hypoallergenic molecules. To address this, we selected parvalbumins from Atlantic salmon (Sal s 1), from tuna (Thu a 1), from chub mackerel (Sco j 1), from herring (Clu h 1) and from codfish (Gad c 1) and expressed the wild-type proteins as well as derivatives mutated in the four conserved amino acids in *Escherichia coli*. Immunoblot analyses with sera from fish allergic patients revealed IgE reactivity to all wild-type parvalbumins, with patient dependent differences in intensity of IgE binding to the individual parvalbumins. In contrast, none of the mutated variants could be recognized by patients' IgE antibodies. These results demonstrate that the four identified amino acids are also involved in the formation of IgE binding epitopes in parvalbumins other than Cyp c 1 and that mutagenesis of these amino acids represents a general strategy for the generation of hypoallergenic fish vaccines.

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Abstracts

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IgE as antitumor adjuvant: Towards a safe human vaccine based on a recombinant MVA encoding for a mini membrane IgE

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Redirection of the IgE-mediated immune system activation to combat tumors is a new research area [1, 2]. Mouse IgE has been shown to provide a potent adjuvant effect in antitumor vaccination [3]. Modified vaccinia virus Ankara (MVA) has been used to infect mouse IgE-loaded tumor cells [4], in the perspective to use recombinant (r) MVA. Loss of IgE adjuvanticity in FcεRIα$^{-/-}$ mice but not in CD23$^{-/-}$ mice attested a crucial role played by FcεRI in the antitumor effect [4]. Most importantly, IgE adjuvanticity was observed also using human IgE in FcεRIα$^{-/-}$ hFcεRIα^{+} mice [4]. These results led to a shift towards a highly safe protocol employing membrane IgE (mIgE). The use of mIgE eliminates any possible anaphylactogenicity caused by circulating IgE. Thus, evidence that human mIgE and a truncated version lacking IgE Fabs (tmIgE) bind and activate FcεRI has been fundamental [5]. Human tmIgE has been engineered into rMVA (rMVA-tmIgE), taking advantage of a new and fast rMVA production strategy named Red-to-Green gene swapping [6, 7]. Expression and transport of tmIgE on the surface of rMVA-tmIgE-infected cells has been detected by Western blot and cytofluorimetry, respectively, and FcεRI activation by tmIgE has been confirmed by the release of β-hexosaminidase in a cell-to-cell contact assay using human FcεRI-transfected RBL-SX38 cells [5]. The rMVA-tmIgE antitumor vaccination strategy is presently being investigated *in vivo*, in FcεRIα$^{-/-}$ hFcεRIα^{+} mice, with preliminary results indicating a level of protection comparable to that obtained using soluble human IgE tumor cell loading. The rMVA-tmIgE vector represents the ultimate molecular device (in which multiple gene insertions should couple tmIgE with tumor-associated antigens or other tumor-related cDNAs) to attain potent, safe IgE-based antitumor vaccines, bringing the field closer to the clinics.

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The effects of immunotherapy on blood dendritic cells in patients with hymenoptera venom allergy

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Background: Animal models suggest that plasmacytoid dendritic cells (pDCs) can suppress allergic immune responses, but the role of pDCs in patients with allergic diseases undergoing immunotherapy is unknown.

Methods: Subcutaneous venom immunotherapy was initiated in 20 patients (median age: 48 years) with hymenoptera venom allergy (18 patients with wasp venom allergy and 2 patients with bee venom allergy) over a period of 52 hours with increasing doses of the allergen. Directly before and one hour after initiation of immunotherapy, the number as well as the expression of function-associated molecules of myeloid DCs (mDCs) and pDCs were analysed in blood using four-colour flow cytometry.

Results: After initiation of immunotherapy, there was a marked decrease in total pDC numbers in peripheral blood of all patients (to a median of 64% of the pDC numbers before initiation of immunotherapy). In contrast, total mDC numbers were not altered in the same time period. After initiation of immunotherapy, blood pDCs were characterised by an increased expression of the tissue-homing receptor CCR-5 and of thrombomodulin (CD141). The effect of 12 months of immunotherapy on the number and the characteristics of blood DCs is currently being studied.

Conclusion: Initiation of immunotherapy results in a specific decrease in circulating pDCs, which appears to reflect a recruitment of this DC subtype to the site of allergen deposition. In addition, immunotherapy has effects on the surface molecule expression of pDCs. Taken together, our preliminary data suggest that pDCs might indeed be involved in the development of immune tolerance in humans.

ORAL ABSTRACT SESSION 14: Emerging Aspects in Allergic Innflammation

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Stressed airway epithelial cells release a nuclear alarmin IL-33 and trigger Th2-type immune responses

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Rationale: Airway epithelial cells constitute the first line of defense against inhaled allergens and play roles in immune regulation. IL-33 is usually localized in nuclei of airway epithelial cells and has been recognized as a key cytokine involved in Th2-type immune responses. This project investigated the mechanisms for IL-33 release by airway epithelial cells and the roles of IL-33 in Th2-type immune responses.

Methods: Normal human bronchial aiway epithelial (NHBE) cells were stimulated with pharmacological and immunological agents as well as extracts of the fungus, *Alternaria alternata,* in vitro. Non-sensitized naïve mice were exposed to *Alternaria* extract in vivo.

Results: NHBE cells exposed to a calcium ionophore, ionomycin, or to *Alternaria* extract, but not those exposed to TLR ligands, released stored IL-33 within 2 hours. *Alternaria* extract induced a sustained increase in intracellular calcium concentration in NHBE cells, and chelation of extracellular calcium by EGTA abolished IL-33 release. LDH measurements and a cell-damage assay suggest that two pathways, namely active- and damage-mediated, are involved in IL-33 release. Furthermore, intranasal administration of *Alternaria* to non-sensitized naïve mice induced rapid IL-33 release (<1 hour), followed by IL-5 and IL-13 production during the next several hours. These IL-5 and IL-13 responses were inhibited by blocking the IL-33 pathway by anti-receptor antibody or Fc-fusion protein.

Conclusions: Activation or damage (or both) of airway epithelial cells by exposure to certain airborne allergens may release IL-33 into the airways, leading to the development of Th2-type immune responses.

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NGF is an essential survival factor for bronchial epithelial cells during respiratory syncytial virus infection

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Background: Overall expression of neurotrophins in the respiratory tract is upregulated in infants infected by the respiratory syncytial virus (RSV), but it is unclear where (structural vs. inflammatory cells, upper vs. lower airways) and why, these changes occur. We analyzed systematically the expression of neurotrophic factors and receptors following RSV infection of human nasal, tracheal, and bronchial epithelial cells, and tested the hypothesis that neurotrophins work as innate survival factors for infected respiratory epithelia.

Methodology: Expression of neurotrophic factors (nerve growth factor, NGF; brain-derived neurotrophic factor, BDNF) and receptors (trkA, trkB, p75) was analyzed at the protein level by immunofluorescence and flow cytometry and at the mRNA level by real-time PCR. Targeted siRNA was utilized to blunt NGF expression, and its effect on virus-induced apoptosis/necrosis was evaluated by flow cytometry following annexin V/7-AAD staining.

Principal Findings: RSV infection was more efficient in cells from more distal (bronchial) vs. more proximal origin. In bronchial cells, RSV infection induced transcript and protein overexpression of NGF and its high-affinity receptor trkA, with concomitant downregulation of the low-affinity p75NTR. In contrast, tracheal cells exhibited an increase in BDNF, trkA and trkB, and nasal cells increased only trkA. RSV-infected bronchial cells transfected with NGF-specific siRNA exhibited decreased trkA and increased p75NTR expression. Furthermore, the survival of bronchial epithelial cells was dramatically decreased when their endogenous NGF supply was depleted prior to RSV infection.

Conclusions/Significance: RSV infection of the distal airway epithelium, but not of the more proximal sections, results in overexpression of NGF and its trkA receptor, while the other p75NTR receptor is markedly downregulated. This pattern of neurotrophin expression confers protection against virus-induced apoptosis, and its inhibition amplifies programmed cell death in the infected bronchial epithelium. Thus, pharmacologic modulation of NGF expression may offer a promising new approach for management of common respiratory infections.

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IL-25 in asthma: A potential role in angiogenesis

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IL-25 (IL-17E) is a Th2 type cytokine best described as a potentiator of Th2 memory responses. Reports of expression of its receptor, IL-25R, on airways structural cells suggest a wider role for IL-25 in remodelling. We hypothesized that IL-25 stimulates local angiogenesis in the asthmatic bronchial mucosa. Immunoreactive IL-25$^+$, IL-25R$^+$ and CD31$^+$ (endothelial) cells in sections of bronchial biopsies from asthmatics and controls were detected by immunohistochemistry. The effect of IL-25 on angiogenesis was examined using an *in vitro* assay. RT-PCR was employed to detect expression of IL-25R and VEGF mRNA in cultured human vascular endothelial cells (HUVEC). Immunohistochemistry showed that IL-25$^+$, IL-25R$^+$ and CD31$^+$/IL-25R$^+$ cells were significantly elevated in the bronchial mucosa of asthmatics compared with controls ($p<0.003$). In the asthmatics, the numbers of IL-25$^+$ cells correlated inversely with FEV_1 ($r=-0.639$, $p=0.01$). *In vitro*, HUVEC constitutively expressed IL-25R which was further upregulated by IL-25 and TNF-α, both of which also increased expression of VEGF and VEGF receptors. In an *in vitro* angiogenesis assay, IL-25 increased the numbers, length and areas of microvessel structures in dose dependent manner. VEGF blockade, the PI3K specific inhibitor LY294002 and the MEK 1/2 specific inhibitor U0126 all markedly attenuated IL-25-induced angiogenesis, while the inhibitors also reduced VEGF expression. Our data suggest that IL-25 is elevated in asthma and contributes to angiogenesis by increasing endothelial cell VEGF/VEGFR expression through PI3K and Erk/MAPK pathways.

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Intestinal mast cell levels determine oral antigen-induced anaphylaxis severity

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Introduction: Severe food allergy-related reactions, termed food-triggered anaphylaxis are serious and life-threatening. Patients do not generally present with a consistent constellation of symptoms and distinction on food-induced anaphylaxis susceptibility and severity cannot necessarily be predicted based on clinical history. The aim of this study is to define biological markers that may distinguish between oral antigen-induced non life threatening and life threatening anaphylaxis.

Methods: Wild-type (WT), FcεRI$^{-/-}$ and intestinal IL-9Tg mice were subjected to passive and active oral antigen (ovalbumin [OVA])-induced anaphylaxis. Severity of anaphylaxis was gauged by intestinal symptoms (secretory diarrhea and intestinal mastocytosis) and systemic symptoms (bronchoconstriction (Penh), hypothermia, intravascular leak and serum mcpt-1). Specific Ig isotypes and αTNP-IgE antibodies were administered prior to challenge to define the role of mast cell load on oral antigen-triggered anaphylaxis.

Results: Repeated oral antigen challenge of OVA-sensitized WT mice induced anaphylaxis with both systemic and intestinal

involvement; both were dependent on IgE/FcεRI/mast cells. Anti-IgE-induced anaphylaxis induced systemic and intestinal symptoms in WT and IL-9Tg mice. Notably, severity of the response was greater in IL-9Tg mice compared with WT mice (ΔTemp (°C) WT -2.8 ± 0.3 vs IL-9Tg -4.9 ± 0.9; $p < 0.05$; Respiratory (Penh) WT: 1.5 ± 0.3 vs IL-9Tg: 2.8 ± 1.0; $p < 0.05$; mean ± SEM; n = 8 and 18). Oral administration of TNP-BSA to WT and IL-9Tg mice administered αTNP-IgE induced oral antigen-triggered anaphylaxis with systemic and intestinal involvement. The intestinal (diarrhea) and systemic (hypothermia) response was significantly greater in IL-9Tg mice compared with WT mice.

Conclusions: Oral antigen-triggered anaphylaxis in mice induces both intestinal and systemic symptoms and is mediated by IgE-dependent pathways. The severity of oral antigen-triggered anaphylaxis in elevated in mice with increased intestinal IL-9 and mast cells. These observations indicate that increased IL-9 and intestinal mast cells may distinguish between life threatening and non-life threatening food-triggered anaphylaxis.

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FcεRI subunit expression pattern: Functional implication in IgE-mediated intestinal disorders

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Background: We have recently reported that the α-chain of the high affinity IgE receptor FcεRI is expressed on human enterocytes in tissue samples from patients with colon cancer or gastrointestinal inflammations, but not from healthy controls. Moreover, also human intestinal tumor cell lines express FcεRIα dependent on the state of differentiation. To reveal possible receptor functions, we aimed to further investigate the subunits expressed in enterocytes.

Methods & results: By immunofluorescence the presence of FcεRI β- and γ-chain was examined in FcεRI α-chain positive intestinal tissue sections from colon cancer or gastrointestinal inflammation patients. A co-expression of FcεRI α- and γ-chain was detected along or at the top of the villi of all small intestinal tissue sections from colon cancer patients and in 3 out of 4 specimens from patients suffering from intestinal inflammation. In the colonic mucosa and in the tumor/lesional area a γ- and α -chain co-expression was found in 66% of the samples. None of the specimens were positive for the β-chain in the epithelial layer, with one exception. The Paneth cells at the base of the crypts expressed prominently FcεRIα, but they revealed neither β- nor γ-chain staining. Additionally, human intestinal cell lines CaCo2 (TC7) and HCT8 expressed a functional FcεRI, comprised of α-chain and γ-chain, only in undifferentiated, subconfluently grown cells. This strongly correlated with the IgE binding capacity of the cells. **Conclusions:** Our data suggest that differences in FcεRI α-chain and γ-chain expression pattern on intestinal cells might determine their function in IgE mediated disease and therefore contribute to the pathophysiology of the gastrointestinal tumors or inflammations.

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ORAL ABSTRACT SESSION 15: Immunotherapies

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TGF-beta and functional regulatory T cells in effective sublingual immunotherapy for house dust mite allergy

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This study aimed to identify the immunological mechanisms underpinning clinically effective sublingual house dust mite immunotherapy using a randomised double blind placebo controlled study.

Background: Despite meta-analyses confirming clinical efficacy and increasing popularity as a therapy, knowledge of the underlying immunological mechanisms of effective sublingual allergen-specific immunotherapy are extremely limited

Methods: Thirty patients with perennial house dust mite allergy were recruited into a 12-month randomised double-blind placebo-controlled study of sublingual house dust mite immunotherapy. One-year open extension was followed in 9 patients on active treatment. Peripheral blood mononuclear cells were stimulated *in vitro* with house dust mite extract and analysed for proliferation, cytokine secretion and regulatory T cells using flow cytometry and ELISA. The effects on proliferation of blocking TGF-beta and IL-10 were determined. Functional T regulatory cell activity was measured using *in vitro* co-culture assays of suppressor function and allergen-specific antibody levels were assayed. Symptom, medication and Juniper quality of life scores were monitored to determine clinical efficacy.

Results: House dust mite-induced $CD4^+$ T cell division and IL-5 production were significantly decreased after 6 and 12 months of active treatment but not placebo. Blocking of TGF-beta effects by addition of sTGF-betaRII prevented immunotherapy-induced suppression of allergen-specific T cell proliferation, maximal at 6 months. Decreased house dust mite-specific $CD4^+$ T cell proliferation with increased secretion of IL-10 and serum Der p 2-specific IgG_4 were maximal at 24 months active treatment. Functional regulatory T cell ($CD4^+CD25^+CD127^{lo}$/$Foxp3^+$) activity was evident by suppression of house dust mite-specific effector T cell ($CD4^+CD25^-CD127^{hi}$) proliferation and cytokine production. Clinical efficacy of house dust mite sublingual immunotherapy was supported by significantly decreased total asthma score, rhinitis symptom score and Juniper quality of life score.

Conclusion: This study established the novel finding that TGF-beta mediates the immunological suppression seen early in clinically-effective sublingual house dust mite immunotherapy in addition to induction of regulatory T cells with suppressor function.

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Targeting the MHC class-II antigen presentation pathway as a novel vaccination strategy for allergy

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Compared to the progress in understanding the immunologic mechanisms underlying allergy, progress in allergen-specific immunotherapy (SIT), the only therapy able to cure allergic diseases, is moderate. Only a minority of the patients choose this treatment mainly because it involves 30-70 doctor visits during 3-5 years. SIT is performed by administration increasing doses of allergen to avoid anaphylactic side-effects. In contrast to a true vaccination which induces strong humoral and cellular immune responses with a few injections, SIT is based on immunomodulation which requires a long time to establish.

We have developed modular antigen translocating (MAT) vaccines to increase antigen presentation through the MHC class-II pathway and investigated the therapeutic potential of a prototype MAT-Fel d 1 applied by intralymphatic injection. The *in vivo* efficacy of MAT-Fel d1 was investigated in a mouse model of allergy with three intralympatic injections of cat extract, Fel d1, or MAT-Fel d1 in biweekly interval. For induction of anaphylaxis mice were challenged intraperitoneally with 20µg cat extract, and whole body temperature drop was measured before and 30 minutes after challenge. A drastic temperature drop of 4.8 °C was detected in pre-sensitized untreated mice, whereas mice treated with cat extract or Fel d1 showed a partial protection (ΔT -3 and -3.3°C). In contrast, mice treated with MAT-Fel d1 showed temperature drop of only 1.8 °C comparable to those of PBS-challenged controls. The robust increase in protection is probably due to an increased production of protective Fel d1-specific IgG2a antibodies.

MAT-Fel d1 was further tested a Phase-I/IIa clinical trial. Twenty cat-allergic patients were randomized in two groups: 12 received three intralymphatic injections with MAT-Fel d1 (1, 3, 10µg) and 8 received alum as placebo. Clinical efficacy was evaluated by conjunctival, nasal and skin provocation tests. All patients were assessed at baseline and after therapy for T-cell proliferation, Fel d1-specific immunoglobulins and cytokine secretion in supernatants of T-cell cultures. No drug related adverse events were observed and MAT-Fel d1-trated patients tolerated much more cat extract in challenges than placebo-treated patients, indicating an efficient therapy.

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Specific immune therapy during pregnancy inhibits allergic sensitization and airway disease in offspring mice

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Background: Immune-modulation by induction of natural regulatory mechanisms such as immunological tolerance might play a pivotal role in upcoming concepts for primary prevention of atopic diseases. Critical periods for allergen sensitization and therefore for prevention strategies are still under intense discussion, but pregnancy appears to be a "window of opportunity" for preventing allergen sensitization of the offspring.

Objective: We investigated the effect of repeated maternal allergen exposure via the airways on later allergen-induced sensitization and airway inflammatory responses in the offspring in a murine model.

Methods: Pregnant BALB/c-mice were exposed to aerosolized Ovalbumin (OVA) three times per week from day 7 of pregnancy until delivery (day 0 of the protocol). Offspring were systemically sensitized by six intraperitoneal injections with OVA between day 21 and day 35 of the protocol prior to repeated airway allergen challenges on days 48, 49 and 50. On day 52 of the protocol, allergen sensitization was determined by serum levels of allergen-specific IgE and Th2 cytokine production by spleen cells cultured with OVA *in vitro*, extend of airway inflammation was assessed by numbers of eosinophils in broncho-alveolar fluids and airway reactivity by whole-body plethysmography *in vivo*.

Results: Compared to maternal placebo exposure, maternal OVA exposure suppressed significantly OVA-specific IgE serum levels, diminished Th2 cytokine production, and inhibited development of allergen-induced airway inflammation and airway hyperreactivity in the OVA-sensitized offspring. The protective effect of maternal OVA exposure on allergen sensitization in the offspring was allergen-specific, long-lasting, and associated with significantly increased allergen-specific *in vitro* production of IFN-γ and IL-10 by spleen mononuclear cells of young mice. Further, maternal OVA exposure was followed by enhanced frequency of $CD25^{+}Foxp3^{+}$ T cells in the offspring, and, moreover, the protective effect of maternal OVA exposure required the presence of regulatory T cells in the offspring.

Conclusion: In our mouse model repeated maternal allergen exposure via the airways prevented later allergen sensitization and allergen-mediated airway inflammatory responses in the offspring by inducing allergen-specific tolerance. Thus, tolerance induction during pregnancy might be a useful approach for primary prevention against allergic diseases.

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Tolerogenic dendritic cells induce CD4+CD25+Foxp3+ T regulatory cell differentiation from CD4+CD25-Foxp3- T effector cells

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Specific allergen-presenting IL-10-differentiated dendritic cells (DC10) induce allergen tolerance in asthmatic mice and thereby ablate airway hyperresponsiveness to methacholine and dramatically attenuate eosinophilic Th2 inflammatory responses to airway allergen challenge. In this process, the animal's pulmonary Th2 T effector cells (T_{EFF}) are displaced by activated $CD4^{+}CD25^{+}Foxp3^{+}$ T cells (T_{REG}). Intestinal dendritic cells promote oral tolerance by inducing antigen-naïve T cells to differentiate into $CD4^{+}CD25^{+}Foxp3^{+}$ T cells (T_{REG}) in a TGFβ-dependent fashion. However, whether DC10 induce tolerance in asthma by simply fostering T_{EFF} stasis or loss together with overgrowth by T_{REG}, or alternately by inducing T_{EFF} to differentiate into T_{REG} has not been explored.

Herein we addressed this question in a mouse model of ovalbumin (OVA)-induced asthma, wherein we treated the animals with specific or irrelevant (house dust mite; HDM) allergen-presenting DC10. OVA-presenting DC10 treatment maximally activated lung

T_{REG} in these animals at 3 wk post-treatment, as determined by upregulation of activation markers (ICOS, PD-1, GITR and LAG3) and in *in vitro* functional assays. This *in vitro* regulatory activity was ≥90% reduced by treatment with anti-IL-10 but not anti-TGFb antibodies. In other experiments, OVA-, but not HDM-, presenting DC10 induced ≈43% of CFSE-labeled $CD25^{-}Foxp3^{-}$ T_{EFF} cells from asthmatic OVA-TCR transgenic (DO11.10 or OTII) mice to differentiate into fully functional tolerogenic $CD25^{+}Foxp3^{+}$ T_{REG}. We recapitulated this *in vivo* using OVA-asthmatic BALB/c mice that were co-injected with OVA- or HDM-presenting DC10 (i.p.) and CFSE-labeled $CD4^{+}CD25^{-}Foxp3^{-}$ T_{EFF} cells (i.v.) from the lungs of asthmatic DO11.10 mice. From ≈7 to 21% of the activated (i.e., dividing) DO11.10 T_{EFF} that were recovered two weeks later from the lungs, lung-draining lymph nodes or spleens of the OVA-DC10 recipients had differentiated into $CD4^{+}CD25^{+}Foxp3^{+}$ T_{REG}, while no CFSE-positive T_{REG} were recovered from the HDM-DC10-treated animals. These data demonstrate that DC10 treatments induce tolerance at least in part by inducing T_{EFF} to differentiate into $CD4^{+}CD25^{+}Foxp3^{+}$ T_{REG}.

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Non-IgE blocking antibodies: Surrogate biomarkers of clinical efficacy to grass pollen immunotherapy

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Background: A consistent immunomodulatory effect of grass pollen-specific immunotherapy has been the elaboration of vigorous non-inflammatory IgG_4 antibody responses. These antibodies show poor correlation with clinical parameters that measure treatment effect.

Objectives: We investigated the relationship between clinical outcome and functional antibody responses following 8 months of immunotherapy. Additionally, we assessed whether allergen-specific IgG_4 titers or functional assays of serum inhibitory non-IgE antibodies such as inhibition of IgE-allergen interactions (IgE-blocking factor) and inhibition of CD23-dependent IgE-facilitated allergen binding (IgE-FAB) may be suitable surrogate markers in predicting clinical response to treatment.

Methods: In an 8-month, dose-response randomized double-blind placebo-controlled study of grass pollen immunotherapy, 221 subjects with severe hayfever received Alutard SQ, *Phleum pratense* 100,000 SQ-U, 10,000 SQ-U or placebo injections. Serum samples collected from patients before treatment, after up-dosing, during the pollen season and at the end of the study were measured for allergen-specific IgG_4 titres and for inhibitory activity of non-IgE antibodies in biologic assays of IgE responses.

Results: A time- and dose-dependent increase in serum inhibitory activity for both the IgE-blocking factor and IgE-FAB was associated with quantitative increases in the grass pollen-specific IgG_4 antibody subclass. A significant, inverse relationship was demonstrated between pre-seasonal serum inhibitory activity and combined symptom and rescue medication scores (IgE-FAB: r=-0.25, p=0.001; IgE-blocking factor: r=-028, p<0.001) whereas this was not observed for immunoreactive IgG4 levels r= - 0.09, p=21). Additionally, changes in functional non-IgE antibody responses accounted for up to 40% of the treatment effect in the first pollen season.

Conclusions: Functional assays of inhibitory non-IgE antibodies and IgE-blocking factor are more useful than immunoreactive IgG4 levels as surrogates of clinical response to immunotherapy. Whether these assays may be used as predictive biomarkers in individual patients requires further investigation.

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Induction of cytolytic CD-4+ T cells to soluble antigens and their potential for antigen-specific immunosuppression

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Cytolytic CD4+ T cells have been described occasionally in various settings, in particular during the course of viral infections, but their precise role and the mechanism by which they are elicited are poorly understood.

We have designed a methodology by which virtually any class II restricted T cell epitope can activate and profoundly alter the phenotype and function of CD4+ T cells, even when such cells are already polarized into Th1, Th2 or Th17 effector cells.

This methodology relies on addition to the T cell epitope of a consensus motif containing a thio-disulfide oxidoreductase activity, so that such motif is located outside of the MHC class II binding cleft. This allows the reduction of disulfide bridges of T cell surface proteins with, as a consequence, a prolongation of the synapse with the APC. This triggers a profound and stable suppression of IL-2 transcription, increased production of IFN-gamma, activation of the transcription of cytolytic proteins including granzymes and soluble FasL, and surface expression of NKG2D and its adaptor molecule DAP10. Overall, CD4+ T cells acquire the capacity to induce apoptosis of APC and of bystander effector CD4+ T cells.

Preclinical evidence of efficacy has now been established in 3 different animal models, allergic asthma, EAE as a model of multiple sclerosis and tolerance to graft. Active immunization with thio-oxidoreductase motif-containing T cell epitopes in adjuvant, or passive transfer of in vitro expanded CD4+ T cells show highly significant efficacy in these 3 models.

Induction of apoptosis of APC and of bystander T cells by antigen-specific CD4+ T cells purposely transformed into potent cytolytic cells could represent a novel strategy to eliminate unwanted immune responses.

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