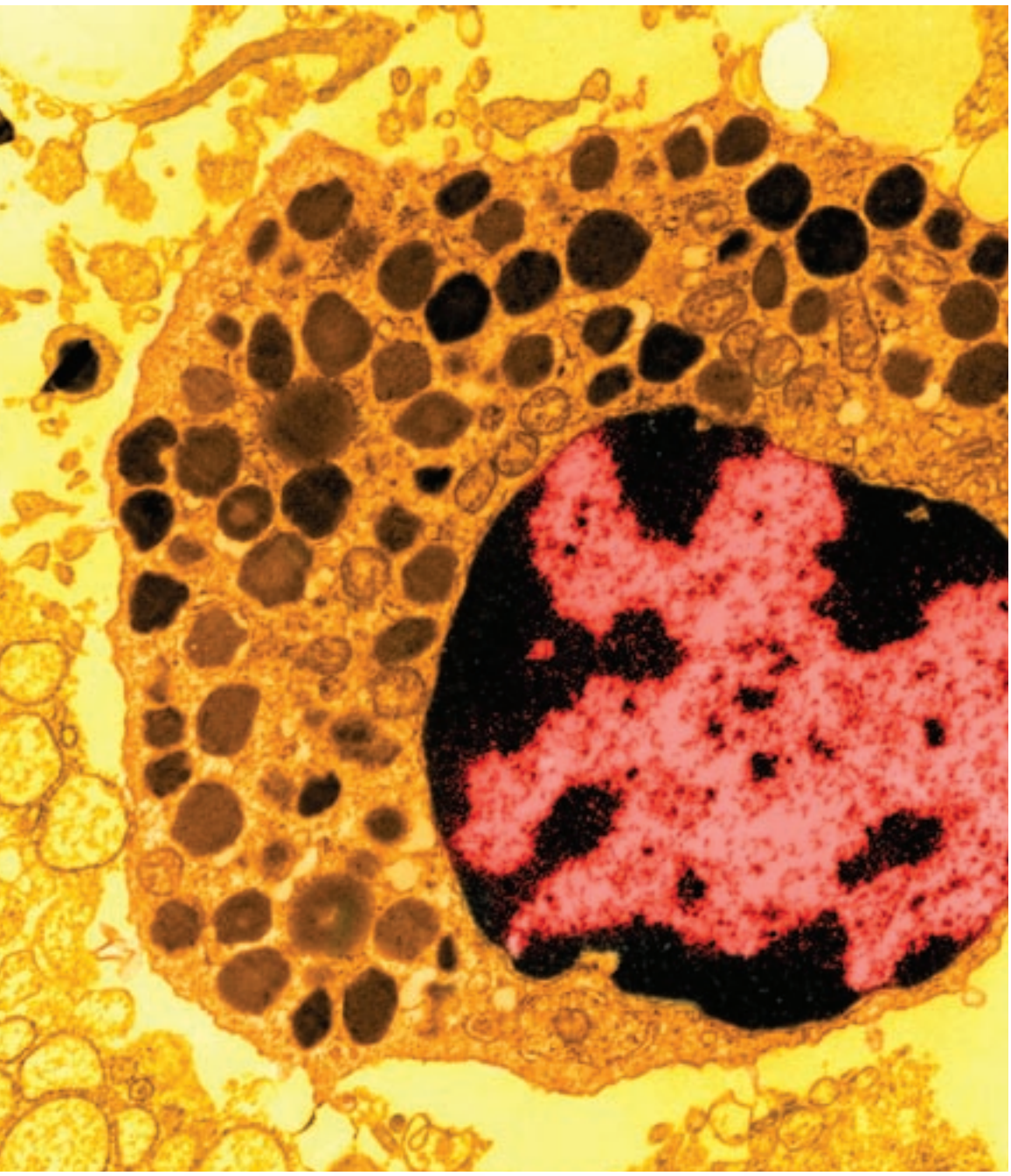
Collegium Internationale Allergologicum

CIA

# 27th Symposium Final Program



*Environmental and Genetic Factors in Allergy and Clinical Immunology*

COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

1-6 May 2008

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# Collegium Internationale Allergologicum

## 27th SYMPOSIUM

CURAÇAO · 1ST – 6TH MAY 2008

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Ronald van Ree
Johannes Ring

Dear colleagues,

On behalf of the *Collegium Internationale Allergologicum*, it is our great pleasure and honor to welcome you to the 27th Symposium in Curaçao. We believe that the program presented over the next week will be both scientifically and socially rewarding.

This Symposium, with over 250 registered attendees and nearly 200 oral and poster presentations, is already a huge success and will surely be remembered by *Collegium* members for years to come.

With enormous research linking both genetic predisposition and environmental factors to allergy and clinical immunology, we have decided to focus the 2008 meeting on "Environmental and Genetic Factors in Allergy and Clinical Immunology." As in previous years, the majority of the scientific program will be given over to free communication 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 are also excited and pleased to offer attendees and guests a great social program and Caribbean hospitality at its best. Guided tours are available for attendees and guests during each day of the meeting. We invite you to discover the remarkable island of Curaçao.

Thank you again for attending the 27th Symposium of the *Collegium Internationale Allergologicum*. 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 Curaçao.

Ronald van Ree
Symposium Organizer

Johannes Ring
Symposium Organizer

Gianni Marone
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# General Information


The 27th Symposium is held on the beautiful island of Curaçao (pronounced kyur uh sow), located just 35 miles north of Venezuela and outside of the hurricane belt.

Curaçao is a truly diverse and remarkable Caribbean island destination. It is best known for its distinctive beaches, outstanding diving, brilliant architecture and multicultural heritage. The island was conqured in 1634 by the Dutch, so many of the Dutch influences are still evident around the island. In addition, Curaçao now represents 55 different nationalities and cultures, each with its own fascinating history, music, dance and cuisine.

## CME

The 27th Symposium of the *Collegium Internationale Allergologicum* will offer 24 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 World Trade Center (WTC), Statue Hall.

## Coffee Breaks

Coffee breaks are included in the registration fee for delegates and will be served daily. Coffee will be served in the World Trade Center; please check the *Schedule of Events* for exact times and locations.

## Currency

The local currency in Curaçao is the Netherlands Antillean Guilder, which is abbreviated as Nafl. U.S. dollars circulate freely, so it is possible to get by using only American dollars or credit cards. Euros are also accepted at some hotels and restaurants, but unlike U.S. dollars, do not circulate freely.

1 USD = Nafl. 1.78
1 EUR = Nafl. 2.72
*(as of April 2008)*

Exchange rates for other currencies are posted at banks and listed in the daily papers. Major credit cards are accepted almost everywhere on the island and ATM machines can be found at various locations.

## Electricity

Electricity is 127/120 VAC at 50 cycles. Curaçao uses the same two-pronged flat plugs as in the United States and most appliances made in the U.S. (60 cycles) will work.

## Evaluations

Evaluation forms are included in the registration packet. Please fill out your form and return it to the Registration Desk in the World Trade Center (WTC), Statue Hall. You must fill out your evaluation in order to receive your CME certificate.

## Government

Curaçao and the neighboring Bonaire, along with three other islands in the eastern Caribbean (St. Maarten, St. Eustatius and Saba), form the Netherlands Antilles, an autonomous part of the Kingdom of the Netherlands. Locals have Dutch nationality and carry European Union passports.

## Hospitality Desk

The Hospitality Desk is located in the lobby of the Marriott. Delegates and accompanying persons will be able to sign up for optional tours and ask for advice regarding activities in Curaçao.

*Hours:*

| | |
|---|---|
| Friday, 2 May: | 7.30 - 11.00 |
| Sunday, 4 May: | 7.30 - 11.00 |
| Monday, 5 May: | 7.30 - 11.00 |

## Lunches

Buffet-style lunches will be served in the Queen's Ballroom at the Curaçao Marriott Beach Resort & Emerald Casino from 13.00 - 15.00 on 2 May, 4 May and 5 May 2008. Lunch is included in the registration fee for both delegates and accompanying persons.

# General Information

## Poster Sessions

Poster presentations are separated by category into six Poster Sessions. Two categories will be presented simultaneously each night. Chairs will introduce the topic and move from one poster to another, allowing each presenter to give a two-minute explanation of their research and for audience members to ask questions.

An assortment of wine and cheese will be served during these sessions, which are scheduled to take place from 17.00 – 19.00 on 2 May, 4 May and 5 May 2008 in the World Trade Center.

Posters Sessions 1, 2, 3 and 5 will take place in the World Trade Center, Exhibition Hall, First Floor and can be set up on the morning of 2 May between 7.00 – 8.00. Poster Sessions 4 and 6 will take place in the World Trade Center, Gallery Hall and can be set up on 3 May between 7.00 – 8.00. Materials for fastening posters to boards will be provided.

## Registration

The Registration Desk is located in the World Trade Center, Statue Hall on all days except Thursday, 1 May, when it is located in the lobby of the Marriott.

*Hours:*

| | |
|---|---|
| Thursday, 1 May: | 14.00 - 22.00 |
| Friday, 2 May: | 7.00 - 13.45 |
| | 16.30 - 20.00 |
| Saturday, 3 May: | 7.30 - 11.15 |
| Sunday, 4 May: | 7.30 - 14.00 |
| | 16.00 - 19.00 |
| Monday, 5 May: | 7.30 - 14.00 |
| | 16.30 - 19.00 |
| Tuesday, 6 May: | 8.30 - 13.00 |

## Registration Fees

*The registration fee for delegates includes:*

Airport Transfers
Coffee Breaks
Lunches
Oral Abstract Sessions
Poster Sessions
Social Events

*The registration fee for accompanying persons includes:*

Airport Transfers
Lunches
Poster Sessions
Relaxing Lecture
Social Events

## Speaker Ready Room

The Speaker Ready Room is located in the World Trade Center, Barber Room. Speakers will be able to check and upload their presentations before the oral sessions.

*Hours:*

| | |
|---|---|
| Friday, 2 May: | 7.00 - 13.15 |
| Saturday, 3 May: | 7.30 - 11.15 |
| Sunday, 4 May: | 7.30 - 13.30 |
| Monday, 5 May: | 7.30 - 13.30 |
| Tuesday, 6 May: | 8.30 - 12.45 |

## Tipping

Tipping in Curaçao is left to your own discretion, but is expected at most locations. It is suggested to tip the porters at the airport Nafl.1 per bag. Taxi-drivers are usually tipped 10% of the fare. Restaurants add 10% and most hotels add a 12% service charge to the bill, but please note that these are not considered tips.

## Transportation

Airport transfers will be arranged for attendees upon arrival and departure. Please confirm your departure pick-up time at the Registration Desk, WTC, Statue Hall.

## Venue

All Oral and Poster Presentations will take place at the Caribbean's premier convention space, the World Trade Center (WTC). Oral Presentations are scheduled in the Auditorium and Poster Sessions will occur in the Exhibition Hall, First Floor and the Gallery Hall. The World Trade Center is conveniently located near the official Symposium hotels, the Curaçao Marriott Beach Resort & Emerald Casino and the Hilton Curaçao.

## Water

The Curaçao water is safe to drink and of the highest quality. In 1928, a seawater distillery for production of drinking water was built in Curaçao. This distillery, now known as Aqualectra, produces soft water that contains no chloride and little calcium. The water is virtually tasteless and odorless and has a good bacteriological composition.

# Social Events

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

## Welcome Reception

**Thursday, 1 May 2008, 20.00 – 23.00**

*Curaçao Marriott Beach Resort & Emerald Casino (pool and ocean view)*

The Welcome Reception will be held poolside at the Curaçao Marriott Beach Resort & Emerald Casino. The pool is set on the ocean's edge, providing a spectacular view for the evening. Musical entertainment will be provided along with open bar and an assortment of hors d'oeuvres.

## Curacao Evenening at Fort Nassau

**Friday, 2 May 2008, 20.00 – 23.00**

On the evening of 2 May 2008, attendees will travel to Fort Nassau. Expect centuries to slide past as you climb the stone stairs and enter an ancient fort that has been transformed into an elegant setting with an unmatched view overlooking Willemstad.

The fort was built in 1797 to defend both the St. Anna Bay as well as part of the city of Willemstad and has been preserved very close to its original state. For years it has served as a harbor signal and control tower, regulating the opening and closing of the Pontoon Bridge.

*Fort Nassau*

*Shuttles will depart from the World Trade Center, Main Entrance at 20.00, immediately following the Relaxing Lecture.*

## Boat Ride to Cas Abou

**Saturday, 3 May 2008, 11.45 – 18.00**

Following in the tradition of past *Collegium* meetings, a boat ride will take place on the third day of the meeting. All Symposium participants will depart from the Curaçao Marriott Beach Resort & Emerald Casino and be taken to the harbor to board the Jonalisa, Miss Ann, Toucan and Pelican. The boats will travel to the Cas Abou beach, where participants will be dropped off for an ocean-side barbeque and various activities.

Cas Abou Beach is located on the southern tip of the island in a quieter spot that is nonetheless popular for its clear blue water and perfect stretch of white sand. The snorkeling and diving here are beyond compare and shade is provided by pergolas and trees. Beach chairs, snorkel gear and other beach activities will be supplied.

*Shuttles will depart from the Marriott Beach Resort and Emerald Casino parking lot at 11.45. Transportation will be provided via shuttle back to the Marriott at 18.00.*

## Gala Dinner at Brakkeput Mei Mei

**Monday, 5 May 2008, 19.15 – 23.00**

An elegant dinner will be served on the last evening of the Symposium at the historic plantation house, Brakkeput Mei Mei, which dates back to the beginning of the 18th Century. The plantation was historically used for raising cattle and to grow corn and beans as a food supply for the cattle and the slaves that worked there. Other products from the plantation, such as fruit, meat and dairy were sold to the town's people and the crew of the ships that docked in the harbor. When the proceeds of the plantation decreased, the burning of lime was used as a source of income.

In 1929, when the last inhabitants of the plantation were no longer able to make a living off the crops, both the house and the land were sold to Koninklijke Shell (Oil Company). In 1985, Brakkeput Mei Mei was placed under the supervision of Stichting Monumentenzorg (Foundation for the Preservation and Restoration of Historic Buildings), and now is used as a restaurant.

*Brakkeput Mei Mei*

*Shuttles will depart from the World Trade Center, Main Entrance at 19.15.*

# Optional Tours

*All tours will depart from the Curaçao Marriott Beach Resort & Emerald Casino Lobby at the times listed below. All half-day tours will return to the Marriott in time for the included lunch. Full day tours will include lunch.*

*To sign up for tours on-site, please visit the Hospitality Desk, located in the lobby of the Marriott Beach Resort and Emerald Casino on Friday, 2 May, Sunday, 4 May and Monday, 5 May from 7.30 – 11.00.*

## Tour Schedule

**Friday, 2 May**

Complete Island Tour

**Saturday, 3 May**

*Boat Ride to Cas Abou*

*No tours offered*

**Sunday, 4 May**

Willemstad Tour

Ostrich Farm and Hato Caves

Jeep Safari

**Monday, 5 May**

Complete Island Tour

Seaworld Explorer

## Complete Island Tour

**Friday, 2 May 2008, 9.00 – 16.00**

**Monday, 5 May 2008, 9.00 – 16.00**

If you are interested in seeing the whole island of Curaçao in one day, this is the tour for you. You will discover that the west and east sides of the island are complete opposites. The east side is well populated and most of the attractions, residential areas and hotels are situated here. Highlights on the east side include the Queen Juliana Bridge, Willemstad, Blue Curaçao Liquor Factory, Scharloo Jewish Neigborhood and the Spanish Waters Bay area.

The west side is defined by hilly landscapes and valleys, a number of unique beach coves, former plantation homes and small fishing villages. Highlights of the west side include West Point, National Christoffel Park, Jaanchi's Restaurant, Boka Tabla Cave, Knip Bay, Flamingo area and the Salt Flats of Jan Kok.

This tour includes lunch.

**Price Per Person: $75.00 USD**

## Willemstad Tour

**Sunday, 4 May 2008, 9.00 – 12.00**

This half-day tour will take participants through the streets of Willemstad, the historical capital of Curaçao and the Netherland Antillies. Willemstad is one of the richest cities in the world in terms of culture and diversity. The city itself is actually divided in two sections—Punda and Otrobanda—connected by the Queen Emma pontoon bridge. Willemstad is a typical port town and was focused on trade with the surrounding colonies, mainly the Spanish colonies on the South American continental coast.

There are 750 historic buildings in Willemstad alone, which the government has determined merit preservation. Progress has been great in the last 10 years and it is likely that, in the very near future, regions of the city will regain their former splendor.

In 1997, the UNESCO World Heritage Site committee selected the historic city center of Willemstad—one of just six Caribbean sites chosen—because of its "outstanding value and integrity, which illustrates the organic growth of a multicultural community over three centuries, and preserves to a high degree significant elements."

**Price Per Person: $48.00 USD**

# Optional Tours

## Jeep Safari

**Sunday, 4 May 2008, 9.00 – 13.00**

The tour will begin with a briefing and safety instructions given by a guide, in reference to driving in open vehicles, specifically in the "wild Curaçao" terrain. Participants will then drive to an off road area, where the 4x4 outdoor adventure will start. Exploring Curaçao's wild nature terrain, with cacti, divi-divi trees, and possible spotting of wara wara birds of pray, lizards, flamingos and wild pigs will certainly be one of the highlights. An included visit to the Brua "VooDoo" cave is an interesting adventure through time.

A refreshing beach break, with the option for a nice swim, will be held at one of Curaçao's beautiful beach coves, where drinks and snacks will be provided.

**Price Per Person: $70.00 USD**

## Ostrich Farm and Hato Caves

**Sunday, 4 May 2008, 9.00 – 13.00**

The tour will begin at the Curaçao Ostrich Farm, which is one of the biggest of its kind outside of Africa, with over 600 birds. Once at the Farm, you will board a Safari Jeep with an expert guide to learn about the Ostrich and feed them with your bare hands. You will see one-day-old chicks to adult birds over 8 years old. After the tour, you will be able to buy plain or decorated ostrich eggs.

The second leg of the tour takes you to the Hato Caves, which were formed below sea level millions of years ago. During the Ice Ages, the water level dropped significantly and Curaçao was born. The Hato Caves covers an area of 4900 $m^2$, and is the home to beautiful limestone formations, romantic pools, waterfalls and a famous Madonna statue.

**Price Per Person: $50.00 USD**

## Seaworld Explorer

**Monday, 5 May 2008, 10.30 – 12.00**

Explore Curaçao's spectacular coral reefs on the Seaworld Explorer, a state-of-the-art semi-submarine. Enjoy this unique, narrated excursion as a diver hand-feeds a multitude of fish in front of your viewing window.

The Seaworld Explorer is a state-of-the-art semi-submarine originally used for research on Australia's Great Barrier Reef. The vessel remains above sea level at all times with passengers seated in the hull, which is five feet below the surface. Huge glass windows provide spectacular views of the many different varieties of corals and fish for which Curaçao is famous. In fact, the fish come right up to your window.

**Price Per Person: $38.00 USD**

**Price Per Child (12 years and younger): $27.00 USD**

# World Trade Center Floor Plan


## Contact Information

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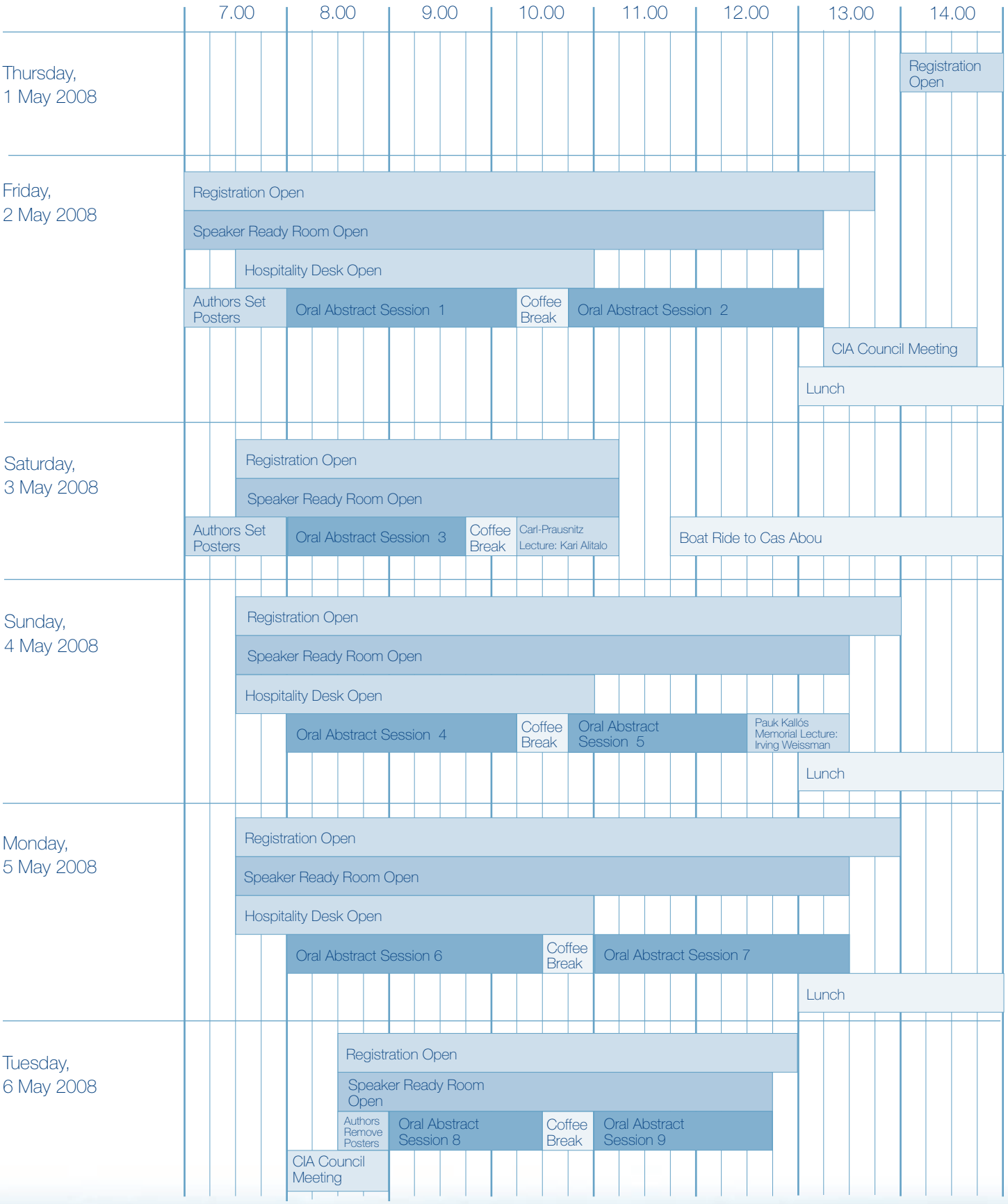
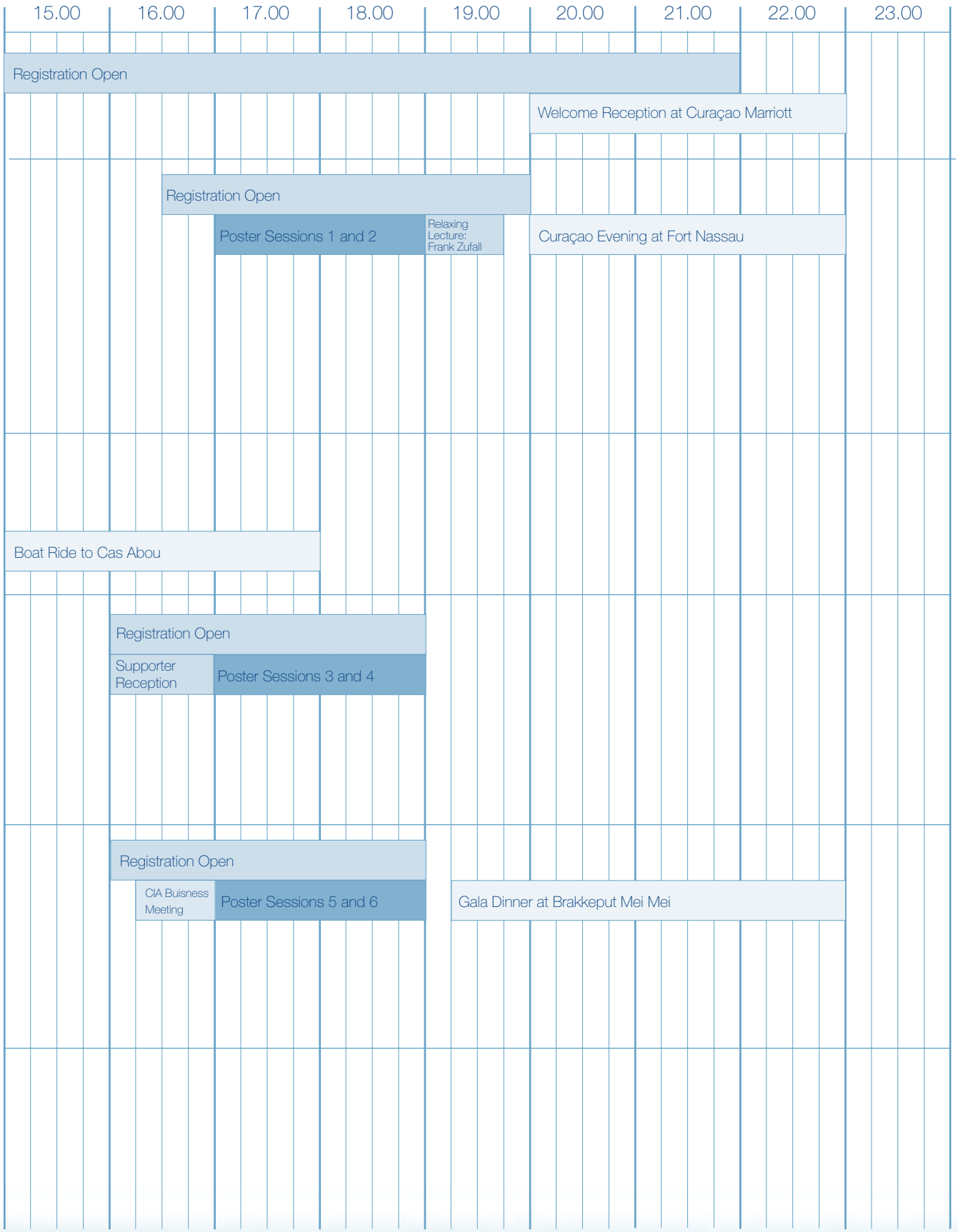
# Program at a Glance



# Program at a Glance



# Schedule of Events

## Thursday, 1 May 2008

| | | |
|---|---|---|
| 14.00 – 22.00 | Registration Open | Marriott Hotel, Lobby |
| 20.00 – 23.00 | **Welcome Reception**<br>Ronald van Ree<br>Johannes Ring | **Marriott Hotel, Poolside** |

## Friday, 2 May 2008

| | | |
|---|---|---|
| 7.00 – 8.00 | Authors Set Posters for Poster Sessions 1, 2, 3, 5 | WTC, Exhibition Hall, First Floor |
| 7.00 – 13.45 | Registration Open | WTC, Statue Hall |
| 7.00 – 13.15 | Speaker Ready Room Open | WTC, Barber Room |
| 8.00 - 8.10 | **Welcome to the 27th Symposium in Curaçao**<br>Gianni Marone | **WTC, Auditorium** |
| 8.10 - 10.15 | **Oral Abstract Session 1:**<br>**Mast Cells and Basophils, Eosinophils and Dendritic Cells 1** | **WTC, Auditorium** |

Chairs: Mariana Castells
Stephen J. Galli

1 Sphingosine-1-phosphate is a key regulator of mast cell responsiveness
*Rivera, Juan*

2 Vascular endothelial growth factors synthesized by human mast cells exert angiogenic, lymphangiogenic, and proinflammatory effects
*Marone, Gianni*

3 Basophils and mast cells play distinct roles in acute and chronic allergic reactions
*Karasuyama, Hajime*

4 The diacylglycerol/phorbol ester-dependent translocation of ras guanine nucleotide-releasing protein 4 (RasGRP4) inside mast cells results in substantial phenotypic changes including the expression of the inhibitory interleukin 13 receptor IL-13Rá2
*Krilis, Steven*

5 Suppression of normal and malignant Kit signaling by a bispecific antibody linking Kit with CD300a
*Levi-Schaffer, Francesca*

6 Induction of mast cell apoptosis – A new way to treat allergy and other mast cell associated diseases?
*Nilsson, Gunnar*

| | | |
|---|---|---|
| 10.15 – 10.45 | Coffee Break | WTC, Exhibition Hall, First Floor |
| 10.45 - 13.15 | **Oral Abstract Session 2:**<br>**Mast Cells and Basophils, Eosinophils and Dendritic Cells 2** | **WTC, Auditorium** |

Chairs: Bruce Bochner
Jean Marshall

7 $Ap_4A$ as a second messenger in a novel gene regulation pathway in immunologically activated mast cells
*Razin, Ehud*

8 Mast cells as modulators of CD8+ T cell responses
*Bulfone-Paus, Silvia*

9 A mite antigen, Der p1, activates dendritic cells (DCs) and stimulates Th2 polarization through a toll-like receptor (TLR)-4 pathway
*Kita, Hirohito*

10 Role of eosinophils in innate immunity
*Capron, Monique*

11 Rapid IgE-antigen desensitization of mouse bone marrow derived mast cells inhibits degranulation, STAT6 phosphorylation and calcium release in an antigen-specific manner
*Castells, Mariana*

# Schedule of Events

## Friday, 2 May 2008 (continued)

12 Expression of cysteinyl leukotriene receptors on human cord blood hemopoietic progenitors: Implications for primary prevention of atopy
*Denburg, Judah*

13 Human eosinophils release glutamate with the potential to modulate survival of a subset of activated T-cells
*Moqbel, Redwan*

13.00 – 15.00 Lunch — Marriott Hotel, Queen's Ballroom

13.15 – 14.45 CIA Council Meeting — WTC, Habaai Room

16.30 – 20.00 Registration Open — WTC, Statue Hall

17.00 – 19.00 **Poster Sessions**

**Poster Session 1: Mast Cells and Basophils, Eosinophils and Dendritic Cells** — **WTC, Exhibition Hall, First Floor**

Chairs: Richard Stevens
Hirohisa Saito

14 Amplification of T helper 1 immunity through CD8 T cell to dendritic cell feedback
*Kemeny, David*

15 Modulating adaptive immune responses with dendritic cells and innate immune receptors
*Pulendran, Bali*

16 Proinflammatory slanDC (6-sulfoLacNAc+ dendritic cells) in atopic dermatitis
*Schäkel, Knut*

17 Regulation of eosinophil function by peroxisome proliferator-activated receptor-γ (PPARγ)
*Ueki, Shigeharu*

18 IgE-mediated activation of human basophils stimulates hypoxia-inducible factor-1 alpha accumulation
*Gibbs, Bernhard*

19 Pleiotropic roles of formyl-peptide receptors expressed on human basophils
*Rossi, Francesca*

20 Are basophils present in skin urticaria lesions?
*Ferrer, Marta*

21 Allergic inflammation: A mast cell - basophil affair?
*Dahinden, Clemens*

22 Retinaldehyde dehydrogenase-II induction and retinoic acid formation in human basophils interacting with IgE-activated mast cells
*Spiegl, Nicole*

23 Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors
*Gurish, Michael*

24 Fibroblasts stimulated by mast cells produce IL-6 that vice versa supports mast cell survival
*Bischoff, Stephan*

25 New genetic mouse models for the investigation of mast cell function in vivo
*Roers, Axel*

26 Virally activated human mast cells induce the selective chemotaxis of NK cells via a CXCL8 dependent mechanism
*Marshall, Jean*

# Schedule of Events

## Friday, 2 May 2008 (continued)

27 TRPC5/Orai1/STIM1-dependent store-operated entry of $Ca^{2+}$ regulates degranulation in a mast cell line
*Beaven, Michael*

28 SNARE proteins in human mast cell degranulation: Role of SNAP23, Syntaxin-4, VAMP-7, and VAMP-8
*Bischoff, Stephan*

29 Activation of protein kinase D1 in mast cells in response to innate, adaptive, and growth factor signals
*Katz, Howard*

30 The role of PI3K for the function of gastrointestinal mast cells
*Koyasu, Shigeo*

31 Expression and release of secretory phospholipases $A_2$ from human lung mast cells
*Triggiani, Massimo*

32 The urokinase/urokinase receptor: A novel target for the treatment of allergic diseases
*Rossi, Guido*

33 Nitration of aldolase: A critical post-translational modification in nitric oxide-mediated regulation of mast cell function?
*Befus, A. Dean*

34 Mast cells as a source of 15-lipoxygenase products
*Kumlin, Maria*

35 Evidence that mast cell degradation of neurotensin contributes to survival in a mouse model of sepsis
*Piliponsky, Adrian M.*

**Poster Session 2: Genetics and Clinical** **WTC, Exhibition Hall, First Floor**

Chairs: Robert Naclerio
Stephen Holgate

36 Gene expression profiling after acute versus chronic allergen airway exposure reveals distinct gene clusters
*Hamelmann, Eckard*

37 Ichthyosis prematurity syndrome: Monogenetic skin disease with concomitant hallmarks of allergy
*Jahnsen, Frode L.*

38 Pharmacogenetic approaches in asthma: Will personalized therapy guide asthma therapy?
*Bleecker, Eugene*

39 Routes of allergic sensitization to peanut protein
*Lack, Gideon*

40 Filaggrin loss-of-function mutations, pet and dust mite exposure and the risk of eczema in childhood
*Simpson, Angela*

41 Endotoxin levels in house dust and early infant gut microbiota
*Jenmalm, Maria*

42 Asthma symptoms among schoolchildren in Georgia: ISAAC Phases I, II and III
*Gamkrelidze, Amiran*

43 Sensitization to ragweed pollen in Switzerland: An emerging problem in the SAPALDIA (Swiss Study on Air Pollution and Lung Diseases in Adults) cohort
*Schmid-Grendelmeier, Peter*

# Schedule of Events

## Friday, 2 May 2008 (continued)

44 Endotoxin augments myeloid dendritic cell influx into the airways in allergic asthma patients
*Krug, Norbert*

45 Asthma deterioration induced by repeated low dose allergen exposure as a model to assess treatment effects on development of allergic airway inflammation
*Dahlén, Barbro*

46 Increase in human neutrophil peptides 1-3 during rhinovirus-induced experimental asthma exacerbations
*Rohde, Gernot*

47 Acute asthma in children presenting to an emergency room - role of infection versus allergy
*Le Souëf, Peter*

48 Nontypable haemophilus influenzae (NTHi): A cause of bronchospasm and a target for therapy in subjects with smoking-related chronic airways disease (SRCAD)
*Clancy, Robert*

49 An intranasal steroid reduces eye symptoms after repetitive nasal challenges with allergen
*Naclerio, Robert*

50 Changes in central nervous processing of histamine-induced itch in atopic eczema
*Darsow, Ulf*

51 Eczema herpeticatum recidivans
*Wollenberg, Andreas*

52 Establishment of food provocation network in Japan
*Ebisawa, Motohiro*

53 When anti-acid treatment goes sour: Food allergy induction in the whole system
*Pali-Schöll, Isabella*

54 Sensitization to Hymenoptera venom in patients with anaphylaxis to Hymenoptera stings and mastocytosis
*Rerinck, Helen-Caroline*

55 Stanozolol therapy for hereditary angioedema
*Sheffer, Albert*

19.00 – 19.45 **Relaxing Lecture** *(Accompanying persons welcome)* **WTC, Auditorium**

**Immunology, Olfaction and Behavior**

**Frank Zufall, PhD**

Chair: Johannes Ring

Frank Zufall was trained in biomedical engineering, biology and physiology and received his PhD from the Technical University of Munich in Germany. He did a postdoc with Gordon Shepherd at Yale University and became Assistant Professor at Yale in 1992. In 1997, he moved to the University of Maryland School of Medicine in Baltimore where he later became a tenured Full Professor. In 2006, he returned to Germany where he is now Professor of Physiology at the University of Saarland School of Medicine. Dr. Zufall's research interests are focused on the mechanisms underlying chemical communication and pheromone sensing in mammals. More recently, he and his collaborators have established that the olfactory system detects vital immune system molecules, with direct consequences for social behavior.

# Schedule of Events

## Friday, 2 May 2008 (continued)

20.00 – 23.00 **Curaçao Evening** **Fort Nassau**

*Shuttles will depart from the World Trade Center (WTC), Main Entrance at 20.00.*

## Saturday, 3 May 2008

7.00 – 8.00 Authors Set Posters for Poster Sessions 4 and 6 WTC, Gallery Hall

7.30 – 11.15 Registration Open WTC, Statue Hall

7.30 – 11.15 Speaker Ready Room Open WTC, Barber Room

8.00 - 9.45 **Oral Abstract Session 3:**
**Genetic and Environmental Factors in Allergic Disorders** **WTC, Auditorium**

Chairs: Heidrun Behrendt
Eugene Bleecker

56 Maternal undernutrition during pregnancy results in persistent alterations in lung gene and miRNA expression: Evidence for fetal programming of lung development
*Holloway, John*

57 Opposite effects of day-care attendance on atopic sensitization and wheezing among children with different polymorphism in TLR2
*Custovic, Adnan*

58 The gene encoding *SMYD3*, a histone methyltransferase, is associated with asthma in ethnically different populations
*Barnes, Kathleen*

59 Genetic pathway analysis in severe asthma
*Meyers, Deborah*

60 Early aberrant antibody responses of aeroallergen-sensitized people to subclinical bacterial infection
*Thomas, Wayne*

9.45 – 10.15 Coffee Break WTC, Statue Hall

10.15 – 11.15 **Carl-Prausnitz Lecture:** **WTC, Auditorium**

**Lymphangiogenesis in Development and Human Disease**

**Kari Alitalo, MD, PhD**

Chair: Gianni Marone

Dr. Kari Alitalo is a tenured Research Professor of the Finnish Academy of Sciences and the Director of the Molecular/Cancer Biology Program and Centre of Excellence in the Biomedicum Helsinki. During his postdoctoral period in 1982-1983 Dr. Alitalo worked with Dr. J. Michael Bishop and his collaborator, Dr. Harold E. Varmus in San Francisco, where he made important contributions to the characterization of the Myc ongogene and -protein. He was furthermore among the first to discover (Myc) oncogene amplification in chromosomal abnormalities of tumor cells. He has also discovered several novel receptor tyrosine kinases, particularly in endothelial cells. He has shown that some of these receptors and their ligands play important roles in tumor angiogenesis. Among the original findings are the cloning and characterization of fibroblast growth factor receptor- 4, the C-terminal Src tyrosine kinase and the first endothelial specific receptor tyrosine kinase, Tie1, as well as VEGFR-3 and the cloning and characterization of VEGF-B in collaboration with Dr. Ulf Eriksson and determination of VEGFR-1 and NP-1 as its receptors.

A significant achievement by Dr. Alitalo was the isolation, cloning and characterization of the first lymphangiogenic growth factor VEGF-C and isolation of lymphatic endothelial cells, opening up the lymphatic

# Schedule of Events

## Saturday, 3 May 2008 (continued)

vascular system to molecular analysis after over a hundred years of descriptive pathology. He has also been central in the characterization of VEGF-B, VEGF-C and VEGF-D receptors and signal transduction pathways and the function of VEGFR-3, showing that this receptor is required for angiogenesis and later in lymphangiogenesis in embryos. He has invented molecular therapies for lymphedema that are now entering clinical trials.
He has furthermore demonstrated that VEGF-C is overexpressed in tumors and its receptor VEGFR-3 is upregulated in angiogenic tumor vasculature. His studies led to the demonstration of VEGF-C associated tumor lymphangiogenesis, intralymphatic tumor growth and tumor metastasis and their inhibition by blocking the VEGFR-3 signal transduction pathway.

11.45 – 18.00 **Boat Ride to Cas Abou**

*Shuttles will depart from the Marriott parking lot at 11.45. Transportation will be provided via shuttle back to the Marriott at 18.00.*

## Sunday, 4 May 2008

7.30 – 14.00 Registration Open — WTC, Statue Hall

7.30 – 13.30 Speaker Ready Room Open — WTC, Barber Room

8.00 - 10.15 **Oral Abstract Session 4: Pathophysiology 1** — **WTC, Auditorium**

Chairs: Ruby Pawankar
Harald Renz

61 Structure and mechanism of human leukotriene $C_4$ synthase, an integral membrane protein involved in bronchial asthma
*Haeggström, Jesper*

62 Leukotriene $E_4$ activates peroxisome proliferator activated receptor gamma and potently induces prostaglandin $D_2$ generation by human mast cells
*Boyce, Joshua*

63 Identification of a critical link between TRAIL and CCL20 for the activation of Th2 cells and the expression of allergic airway disease
*Foster, Paul*

64 Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease
*Izuhara, Kenji*

65 The mast cell-restricted, tetramer-forming tryptases mMCP-6 and mMCP-7 are critical mediators in a mouse model of inflammatory arthritis
*Stevens, Richard*

66 Interfering with histamine-mediated signaling results in significant protection against severe malaria in mice
*Mecheri, Salaheddine*

10.15 – 10.45 Coffee Break — WTC, Statue Hall

10.45 – 12.30 **Oral Abstract Session 5: Cells of Immunoregulation** — **WTC, Auditorium**

Chairs: Massimo Triggiani
Hans Merk

67 Age related changes in regulatory T cell markers and toll-like receptor (TLR)-mediated innate mediated responses in allergic and nonallergic children
*Prescott, Susan*

# Schedule of Events

## Sunday, 4 May 2008 (continued)

68 Mechanisms of direct T cell suppression by IL-10 and TGF-beta
*Akdis, Cezmi*

69 Identification of IL-17RB+ NKT cells preferentially producing IL-13 as a novel subset responsible for development of AHR
*Taniguchi, Masaru*

70 Clonal switch to IL-10-secreting Type 1 T regulatory cells in high dose allergen exposure
*Akdis, Mübeccel*

71 A role for T cell independent interleukin 10 in allergen immunotherapy
*Jakob, Thilo*

12.30 – 13.30 **Paul Kallós Memorial Lecture:** **WTC, Auditorium**

**Normal and Neoplastic Stem Cells**

**Irving L. Weissman, MD**

Chair: Stephen J. Galli

Irving L. Weissman, MD, is the Director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, Director of the Stanford Cancer Center and Director of the Stanford Ludwig Center for Stem Cell Research. Dr. Weissman was a member of the founding Scientific Advisory Boards of Amgen (1981-1989), DNAX (1981- 1992), and T-Cell Sciences (1988-1992). He co-founded SyStemix in 1988, StemCells in 1996, and Celtrans (now Cellerant), the successor to SyStemix, in 2001. He is a Director and Chair of their Scientific Advisory Boards.

His research encompasses the biology and evolution of stem cells and progenitor cells, mainly blood forming and brainforming. He is also engaged in isolating and characterizing the rare cancer and leukemia stem cells as the only dangerous cells in these malignancies, especially with human cancers. Finally, he has a long-term research interest in the phylogeny and developmental biology of the cells that make up the bloodforming and immune systems. His laboratory was first to identify and isolate the blood forming stem cell from mice, and has purified each progenitor in the stages of development between the stem cells and mature progeny (granulocytes, macrophages, etc.). At SyStemix he co-discovered the human hematopoietic stem cell and at StemCells, he co-discovered a human central nervous system stem cell. In addition, the Weissman laboratory has pioneered the study of the genes and proteins involved in cell adhesion events required for lymphocyte homing to lymphoid organs in vivo, either as a normal function or as events involved in malignant leukemic metastases.

13.00 – 15.00 Lunch — Marriott Hotel, Queen's Ballroom

16.00 – 19.00 Registration Open — WTC, Statue Hall

16.00 – 17.00 Supporter Reception — WTC, Gallery Hall

17.00 – 19.00 **Poster Sessions**

**Poster Session 3: Pathophysiology and Immunoregulation 1** **WTC, Exhibition Hall, First Floor**

Chairs: Mübeccel Akdis
Michael Beaven

72 The "transistor model" of T cell differentiation
*Schmidt-Weber, Carsten*

73 CD4+ T cells with regulatory characteristics and effector functions: A step beyond regulatory T cells
*Saint-Remy, Jean-Marie*

74 CD4+T cells from atopic dermatitis patients have differential expression of genes related to T cell homing proliferation and apoptosis mainly due to selective expression in the skin homing T cells
*Knol, Edward*

# Schedule of Events

## Sunday, 4 May 2008 (continued)

75 Towards a better characterization of the 'allergen-specific synapse': Molecular and functional analysis of the antigen receptor of Art v 1-specific helper T lymphocytes
*Pickl, Winfried*

76 Chemokine receptor expression by lung T cells in asthma
*Wardlaw, Andrew*

77 Airways remodelling activity of the resistin-like molecule (RELM)-β) in asthmatic airways
*Corrigan, Chris*

78 Mouse models for Kit mutations and mastocytosis
*Hartmann, Karin*

79 IL-31 receptor alpha expression in epidermal keratinocytes is modulated by cell differentiation
*Baron, Jens*

80 Comparison between human neutrophils and eosinophils in matrix metalloproteinase-9 release
*Takafuji, Shigeru*

81 Protease-Activated Receptor-2 (PAR-2) activation mediates allergic sensitization to cockroach extracts
*Vliagoftis, Harissios*

82 Expression and cellular provenance of thymic stromal lymphopoietin (TSLP) and chemokines in patients with severe asthma, COPD and controls
*Sun, Ying*

83 IgE against Staphylococcus aureus enterotoxins: Relevance to disease expression and severity in asthma
*Howarth, Peter*

84 Airway allergen exposure during respiratory viral infections can induce asthma by both Th1 and Th2 immune responses
*Kim, You-Young*

85 In vitro corticosteroid treatment enhances TNF-α-mediated inflammatory reactions of pulmonary microvascular endothelial cells
*Orihara, Kanami*

86 Nitric oxide and ceramide pathway interaction: A new target in allergic asthma
*Masini, Emanuela*

87 Interactions between toll like receptor-3 (TLR-3) and IgE dependent pathways in human lung tissue explants
*Warner, Jane*

88 The nasal mucosa is an important site for induction, maintenance and therapy of systemic allergy
*Niederberger, Verena*

89 Induction of allergic airway inflammation by house dust mite allergen specific Th2 cells in mice
*Chua, Kaw Yan*

90 Regulation of intraepithelial accumulation of mast cells in the nasal mucosa of allergic rhinitics
*Pawankar, Ruby*

91 Non-IgE mediated chronic allergic skin inflammation revealed with rBet v 1 fragments
*Campana, Raffaela*

# Schedule of Events

## Sunday, 4 May 2008 (continued)

**Poster Session 4: Allergens and Therapy 1** **WTC, Gallery Hall**

Chairs: Ronald van Ree
Fatima Ferreira

92 The use of recombinant allergens provides improved solutions for patients with insect venom allergy
*Ollert, Markus*

93 Microarray of allergenic components-based diagnosis in polysensititized patients
*Sanz, María*

94 Utility of a new solid phase multiplex technology to determine patient sIgE responses to whole milk extracts and specific allergenic proteins simultaneously
*Zychlinsky, Emi*

95 Identification and characterization of lentil sensitization patterns
*Akkerdaas, Jaap*

96 Sensitization to Ascaris, Dermatophagoides pteronyssinus and Blomia tropicalis in the tropical Island of Martinique
*Fernández-Caldas, Enrique*

97 Allergen specific IgG antibody levels modify the relationship between allergen specific IgE and current asthma
*Söderström, Lars*

98 Diagnosing hypersensitivity reactions to cephalosporins in children
*Romano, Antonino*

99 The new GA²LEN Pan-European standard prick test: First results
*Zuberbier, Torsten*

100 Characterization of monoclonal antibodies against Bet v 1 and their use as tools for quality assessment of recombinant Bet v 1 derivatives
*Cromwell, Oliver*

101 The evolution of allergenicity in the prolamin and Bet v 1 superfamilies
*Breiteneder, Heimo*

102 Interaction of the major grass pollen allergen Phl p 1 with the respiratory interphase: Activation, uptake and transport
*Petersen, Arnd*

103 Has Ambrosia sensitization any triggering effect on allergies?
*Nekam, Kristof*

104 Genomics of Aspergillus fumigatus allergens
*Crameri, Reto*

105 Doxycycline reduces MMP9, ECP, MPO and nasal polyp size, in a double-blind, randomized, placebo controlled, multicenter trial
*Van Zele, Thibaut*

106 The effects of cetirizine and its enantiomers on the transport of monoamines by human organic cation transporter 2 (hOCT2)
*Whomsley, Rhys*

# Schedule of Events

## Sunday, 4 May 2008 (continued)

107 Targeting the extracellular membrane-proximal domain of membrane-bound IgE by passive immunization blocks IgE synthesis in vivo
*Achatz, Gernot*

108 A specific mixture of short chain galacto-oligosaccharides and long chain fructo-oligosaccharides induces an anti-allergic immunoglobulin profile in infants at risk for allergy
*Knippels, Leon*

109 Combination vaccines for rhinovirus infections and allergy
*Valenta, Rudolf*

110 Patterns of IgE, IgG, skin tests responses to Bet v 1 and birch pollen extract after immunotherapy with recombinant Bet v 1
*Pauli, Gabrielle*

111 Phleum pratense alone is representative of Pooideae grass species for allergen specific immunotherapy
*Larsen, Jorgen*

## Monday, 5 May 2008

| | | |
|---|---|---|
| 7.30 – 14.00 | Registration Open | WTC, Statue Hall |
| 7.30 – 13.30 | Speaker Ready Room Open | WTC, Barber Room |
| 8.00 - 10.30 | **Oral Abstract Session 6: Allergens and Diagnostics** | **WTC, Auditorium** |

Chairs: Henning Løwenstein
Rudolf Valenta

112 Assessment of the clinical relevance of plant-glycan specific IgE by in vivo challenge with transgenic human lactoferrin produced in rice
*van Ree, Ronald*

113 Role of IgE affinity and clonality for basophil activation and facilitated antigen presentation
*Lund, Kaare*

114 Hazelnut oleosin: Identification and characterization of a novel hazelnut allergen
*Zuidmeer, Laurian*

115 Almond and walnut 60S ribosomal protein P2: A new class of IgE-binding food protein with fungal aeroallergen cross-reactivity
*Roux, Kenneth*

116 X-ray crystallography of a monoclonal antibody complex with cockroach allergen Bla g 2 and identification of putative IgE epitopes
*Pomés, Anna*

117 Common traits and individualized patterns in the human IgE repertoire
*Mempel, Martin*

118 Robust long-term tolerance in a murine model of Type I allergy through transplantation of genetically modified hematopoietic stem cells
*Baranyi, Ulrike*

| | | |
|---|---|---|
| 10.30 – 11.00 | Coffee Break | WTC, Statue Hall |

# Schedule of Events

## Monday, 5 May 2008 (continued)

11.00 – 13.30 **Oral Abstract Session 7: Clinical Aspects of Allergic Disorders** **WTC, Auditorium**

Chairs: Oscar Frick
Albert Sheffer

119 Idiopathic anaphylaxis in mast cell clonal disorders is potentially linked to D816V kit-induced hyper-activation of key signaling events for mast cell activation
*Metcalfe, Dean*

120 Disrupted tight junctions and epithelial susceptibility in asthma
*Holgate, Stephen*

121 Expression of calcitonin gene-related peptide in allergic tissue reactions
*Kay, A. Barry*

122 Anaphylaxis in patients with IgE ab to Galactose alpha-1-3 Galactose, can occur on exposure to mammalian proteins (including the monoclonal ab Cetuximab) which are glycosylated with this major xenoantigen
*Platts-Mills, Thomas*

123 T-cells and eosinophils are crucial in the remodeling of bronchial smooth muscle cell layer in asthma
*Jutel, Marek*

124 A new twist in the pharmacology of non-steroidal anti-inflammatory drugs
*Dahlén, Sven-Erik*

125 Autoallergy – a novel pathomechanism in chronic urticaria
*Maurer, Marcus*

13.00 – 15.00 Lunch — Marriott Hotel, Queen's Ballroom

16.00 – 19.00 Registration Open — WTC, Statue Hall

16.15 – 17.00 **CIA Business Meeting** (all CIA Members welcome) — **WTC, Auditorium**

17.00 – 19.00 **Poster Sessions**

**Poster Session 5: Pathophysiology and Immunoregulation 2** — **WTC, Exhibition Hall, First Floor**

Chairs: Stephan Bischoff
Kurt Blaser

126 Th1 / T Reg adjuvants for sublingual allergy vaccines
*Moingeon, Philippe*

127 HLA-classII/peptide-TCR interactions of the single immunodominant T cell epitope of Art v 1, the major mugwort pollen allergen
*Jahn-Schmid, Beatrice*

128 Effects of corticosteroids on mucosal tolerance and on the development of human T cell subsets
*Stock, Philippe*

129 Chronic interleukin (IL)-13 stimulation of human airway epithelial cells alters the chemokine response to rhinovirus (RV)16
*Wenzel, Sally*

130 Cytokine modulation by glutaraldehyde-polymerized ragweed allergen
*Khanferyan, Roman*

131 The CH3 domain rather than the core hinge is primarily involved in IgG4 Fab arm exchange
*den Bleker, Tamara*

132 Endogenous glycan ligands for siglec-8 and siglec-f
*Bochner, Bruce*

# Schedule of Events

## Monday, 5 May 2008 (continued)

133 The role of IL-25 in airways inflammation and remodeling in asthma
*Meng, Qui*

134 Mechanisms of protection from allergies early in life
*Renz, Harald*

135 Receptor revision of B cells in the nasal mucosa contributes to the immunopathogenesis of allergic disease
*Takhar, Pooja*

136 B cells precursors exist in the lung after allergen exposure in a murine model of allergic airway inflammation
*Bossios, Apostolos*

137 Human enterocytes express FcεRI: Effector cell candidates in IgE-mediated intestinal disorders
*Untersmayr, Eva*

138 IgG4 antibodies do not prevent the subsequent development of IgE antibodies. A study in a cohort of apprentice laboratory animal workers
*Aalberse, Rob*

139 T cell-independence of secondary IgE responses in allergy
*Linhart, Birgit*

140 Keratinocytes as immunoregulators during inflammatory skin reactions
*Traidl-Hoffmann, Claudia*

141 Exploring allergic responses in the skin of humans in vivo using laser imaging, dermal microdialysis and skin biopsies
*Church, Martin*

142 Intracellular cAMP-elevating agents enhance thymic stromal lymphopoietin production by airway tissue-derived cells
*Saito, Hirohisa*

143 Adoptive transfer of Th clone conferred asthma phenotypes including airway obstruction
*Mori, Akio*

144 Establishment of severe allergic asthma and dermatitis models caused by repeated application of Dermatophagoides farinae
*Nagai, Hiroichi*

145 Immunoglobulin free light chains are present in chronic inflammatory diseases: Studies in allergic and non-allergic rhinitis, multiple sclerosis and rheumatoid arthritis
*Redegeld, Frank*

146 Mucosal allergen challenge induces therapy-resistant, long-lived IgE plasma cells
*Worm, Margitta*

**Poster Session 6: Allergens and Therapy 2** **WTC, Gallery Hall**

Chairs: Martin Chapman
Marianne van Hage

147 Monitoring cat allergy in childhood by using the major cat allergen, rFel d 1
*van Hage, Marianne*

148 Lipid transfer protein (LTP) and profilin have opposite effects in the clinical reactivity to apple in allergic patients from Spain
*Fernandez-Rivas, Montserrat*

# Schedule of Events

## Monday, 5 May 2008 (continued)

149 Component Resolved Diagnostics in apple and pear allergy across Europe
*Lidholm, Jonas*

150 Component resolve diagnosis in every day allergy practice
*García, Blanca*

151 Prediction of allergic patients' phenotypes with recombinant pollen marker allergens
*Swoboda, Ines*

152 Use of recombinant allergens for diagnosis of mite and cockroach allergy in Brazilian children
*Arruda, L. Karla*

153 Cross-reactive carbohydrate determinants and in vitro allergy diagnostics: Comparison of three conceptionally different automated allergen-specific IgE-detection systems
*Jappe, Uta*

154 Differentiating between true peanut allergy and asymptomatic sensitization to peanut using microarray and component-resolved diagnostics
*Poorafshar, Maryam*

155 Specific IgE measurements by microchips: Theoretical considerations and a comparison between the ISAC®-chip and the immunoCAP
*Poulsen, Lars*

156 Fluorescent multiplex arrays – new tools for the study of gene-environment interactions
*King, Eva*

157 Characterization of naturally processed and presented T cell epitopes of Bet v 1, the major allergen from birch pollen
*Ferreira, Fatima*

158 Bet v 1 concentrations in ambient air do not correlate with birch pollen counts
*Buters, Jeroen*

159 Der p1; a major allergen from Dermatophagoides pteronyssinus, but is there a biological function for Der p1?
*van der Graaf, Kees*

160 Relevance of mite sensitization in workers exposed to grain dust
*Raulf-Heimsoth, Monika*

161 Decelerated differentiation and altered functional properties of cord blood $CD34^{+}$-derived Dendritic cells in newborns at risk for atopy
*Novak, Natalija*

162 Interleukin-10-differentiated human dendritic cells tolerize Th2 responses of atopic subjects by inducing the differentiation of regulatory T cells
*Gordon, John*

163 Grass tablet sublingual immunotherapy (SLIT) for the treatment of allergic rhinoconjunctivitis
*Nolte, Hendrik*

164 Safety and efficacy of oral immunotherapy with a microencapsulated ragweed pollen extract (MRPE) in patients with ragweed-induced seasonal allergic rhinitis (SAR)
*Creticos, Peter*

165 Sublingual latex immunotherapy: Safety and efficacy in anaesthesiologist with latex professional exposure
*Patella, Vincenzo*

# Schedule of Events

## Monday, 5 May 2008 (continued)

166 Vaccination with a modified vaccinia virus ankara-based vaccine prevents allergen-specific sensitization
*Reese, Gerald*

167 Mepolizumab, a humanised anti-IL-5 monoclonal antibody, as treatment of severe nasal polyposis
*Gevaert, Philippe*

168 Lung function at 10 years is not improved by early corticosteroid treatment in asthmatic children
*Lødrup Carlsen, Karin*

19.15 – 23.00 **Gala Dinner** **Brakkeput Mei Mei**

*Shuttles will depart from the WTC, Main Entrance at 19.15.*

## Tuesday, 6 May 2008

| | | |
|---|---|---|
| 8.00 – 9.00 | CIA Council Meeting | WTC, Habaai Room |
| 8.30 – 9.00 | Authors Remove Posters | WTC, Exhibition Hall, First Floor and Gallery Hall |
| 8.30 – 13.00 | Registration Open | WTC, Statue Hall |
| 8.30 – 12.45 | Speaker Ready Room Open | WTC, Barber Room |
| 9.00 - 10.30 | **Oral Abstract Session 8: Pathophysiology 2** | **WTC, Auditorium** |

Chairs: Akio Mori
Dean Befus

169 Expression of functionally active Fcε and Fcγ receptors on mouse superior cervical ganglion neuritis
*Van der Kleij, Hanneke*

170 Activation of sensory nerves by a prostaglandin D2 metabolite
*Undem, Bradley*

171 Genetic communication between mamallian cells via exosomal shuttle of RNA (esRNA)
*Lotvall, Jan*

172 Inducing an anergic state in mast cells and basophils without secretion: Theoretical and practical implications
*MacGlashan, Donald*

| | | |
|---|---|---|
| 10.30 – 11.00 | Coffee Break | WTC, Statue Hall |
| 11.00 - 12.45 | **Oral Abstract Session 9: Therapy** | **WTC, Auditorium** |

Chairs: Cezmi Akdis
Gianni Marone

173 Allergen-S-layer fusion proteins: Promising candidate vaccines for specific immunotherapy of Type I allergies
*Bohle, Barbara*

174 An IgE-based polyplex allergen gene vaccine that targets dendritic cells as a novel therapuetic approach for severe food allergy
*Saxon, Andrew*

175 GATA-3-specific DNAzyme - a novel approach toward asthma therapy
*Garn, Holger*

176 News from the mimotopes: Therapeutic tools against allergy and cancer
*Jensen-Jarolim, Erika*

177 Anti-IgE in servere atopic eczema with high serum IgE levels
*Ring, Johannes*

# Abstracts

## 1
### Sphingosine-1-phosphate is a key regulator of mast cell responsiveness

Juan Rivera, Anastassia Tikhonova, Laura Ciaccia, Sandra Odom, Sandra Dillahunt, Shantelle Lucas, Kiyomi Mizugishi, Richard L. Proia, and Ana Olivera.

Laboratory of Immune Cell Signaling, NIAMS, & Genetics of Development and Disease Branch, NIDDK, NIH, Bethesda, MD, 20892.

Mast cells homeostasis is controlled by specific molecular events that determine whether a quiescent or activated state is necessary. Dysequilibrium of these molecular controls can cause detrimental outcomes that may manifest as disease. Multiple regulatory events preceding and following engagement of the high affinity IgE receptor (FcεRI) are response determinants. Sphingosine-1-phosphate (S1P), a key mediator in immune cell trafficking, is elevated in the lungs of asthmatic patients and regulates pulmonary epithelium permeability. Allergically stimulated mast cells induce two mammalian sphingosine kinases (SphK1 and SphK2) to produce S1P. Activation of these kinases requires the two Src family kinases Lyn and Fyn, but whereas Lyn-deficiency can be overcome, Fyn is indispensable. In activated mast cells, SphK2 is required for production of S1P, calcium influx, activation of protein kinase C, and for cytokine production and degranulation. In contrast, the absence of SphK1 in mast cells had little effect on S1P production, signaling and responses. S1P production results in the transactivation of two mast cell receptors for this ligand ($S1P_1$ and $S1P_2$) that function in chemotaxis and degranulation, respectively. *In vivo* studies showed that the levels of circulating S1P highly correlated with histamine release in wild type mice that had undergone a passive systemic anaphylactic challenge. Challenge of SphK1-null mice, which had low levels of circulating S1P, revealed a reduced allergic response. In contrast, anaphylactic challenge of mice deficient in SphK2, which had enhanced levels of circulating S1P, showed normal allergic responses. Mice null for SphK2 and heterozygous for SphK1 had normal levels of circulating S1P and a defective anaphylactic response, demonstrating an intrinsic role for SphK2. Thus, susceptibility to *in vivo* anaphylaxis is determined by both S1P within the mast cell compartment (generated by SphK2) and circulating S1P (generated by SphK1). The collective impact is exquisite control of the resting and activated state of the mast cell.

## 2
### Vascular Endothelial Growth Factors Synthesized by Human Mast Cells Exert Angiogenic, Lymphangiogenic, and Proinflammatory Effects

Gianni Marone*, Aikaterini Detoraki*, Rosaria I. Staiano*, Francescopaolo Granata*, Giorgio Giannattasio*, Nella Prevete*, Amato de Paulis*, Domenico Ribatti†, Arturo Genovese*, and Massimo Triggiani*

* Department of Clinical Immunology and Allergy and Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy

†Department of Anatomy and Histology, University of Bari, Bari, Italy

Angiogenesis and lymphangiogenesis are multistep complex phenomena critical for several inflammatory and neoplastic disorders. Mast cells infiltrate the sites of chronic inflammation and several tumors. We have characterized the expression and functions of vascular endothelial growth factors (VEGFs) and their receptors in primary human lung mast cells (HLMC), and two human mast cell lines LAD-2 cells and HMC-1. These cells constitutively express mRNA for three isoforms of VEGF-A (121, 165, and 189), two isoforms of VEGF-B (167 and 186), VEGF-C and VEGF-D. Only HMC-1 express mRNA for PlGF-1 and -2. Mast cells contain VEGF-A (28 to 105 pg/$10^6$ cells), which was spontaneously released. $PGE_2$ and an adenosine analog (NECA) significantly enhanced VEGF-A release. Both spontaneous and induced VEGF-A release was blocked by actinomycin D, cycloheximide and brefeldin A. Supernatants of $PGE_2$- and NECA-activated mast cells induced an angiogenic response in the chick embryo chorioallantoic membrane that was inhibited by an anti-VEGF-A antibody. Mast cells expressed mRNA for the tyrosine kinase VEGF receptor-1 (VEGFR-1), the soluble form of VEGFR-1 (sVEGFR-1), and VEGFR-2. VEGF-$A_{165}$, VEGF-$B_{167}$, VEGF-C, VEGF-D and PlGF-1 induced mast cell chemotaxis. These chemotactic effects were mediated by the activation of VEGFR-1 and/or VEGFR-2. Our data suggest that human mast cells play a role in angiogenesis, lymphangiogenesis, and inflammation through the expression of several forms of VEGF and their receptors.

## 3
### Basophils and mast cells play distinct roles in acute and chronic allergic reactions

Karasuyama H[1], Tsujimura Y[2], Obata K[2], Mukai K[2], Shindou H[3], Shimizu T[3], Minegishi Y[2]

[1]Immune Regulation, Tokyo Medical and Dental University Graduate School, Tokyo, Japan; [2]Tokyo Medical and Dental University Graduate School, Tokyo, Japan; [3]The University of Tokyo, Tokyo, Japan

Basophils represent less than 1% of peripheral blood leukocytes, and share several features with tissue-resident mast cells, such as the surface expression of the high-affinity IgE receptor FcεRI, and the release of chemical mediators upon stimulation. Therefore, basophils are often considered to be minor and possibly redundant 'circulating mast cells'. We recently demonstrated that basophils play a pivotal and non-redundant role in the development of IgE-mediated chronic cutaneous allergic inflammation, independently of T cells and mast cells. Here we show that treatment of mice with a newly established, basophil-depleting mAb, Ba103, prior to the antigen challenge completely abolished IgE-mediated chronic allergic inflammation. Notably, even after the skin inflammation became prominent, the Ba103 treatment was still effective and resulted in decreased numbers of eosinophils and neutrophils in the skin lesions, concomitant with elimination of basophils that accounted for only 2% of infiltrates. In response to antigen stimulation, basophils secreted a panel of chemokines and cytokines that stimulated other cells in the skin to produce chemokines necessary for recruitment of inflammatory cells such as neutrophils and eosinophils. These findings indicate a novel mechanism of development of chronic allergic inflammation in which basophils function as initiators rather than effectors.

We next examined the possible role of basophils in immediate-type allergic reactions. It has long been thought that anaphylaxis is mediated by histamine released by mast cells and perhaps basophils upon allergen-induced cross-linking of IgE receptors. However, recent studies suggested that an alternative pathway involving IgG is more important than the classical pathway for anaphylaxis. Here we show, in a mouse model of penicillin shock, that *in vivo* depletion of basophils but not macrophages, neutrophils, platelets, or NK cells ameliorated IgG1-mediated anaphylaxis, and PAF antagonists but not anti-histamine inhibited it. Upon capture of IgG1-allergen complexes via IgG receptors, basophils released PAF, which in turn stimulated endothelial cells to increase vascular permeability. These results highlight a pivotal and non-redundant role for basophils *in vivo*, and demonstrate two major, distinct pathways leading to allergen-induced systemic anaphylaxis: one mediated by IgG, basophils, and PAF; the other by IgE, mast cells, and histamine.

## 4
### The Diacylglycerol/Phorbol Ester-Dependent Translocation of Ras Guanine Nucleotide-Releasing Protein 4 (RasGRP4) Inside Mast Cells Results in Substantial Phenotypic Changes Including the Expression of the Inhibitory Interleukin 13 Receptor IL-13Rá2

Katsoulotos GP[1], Qi M[1], Qi JC[1], Tanaka K[1], Hughes WE[2], Molloy TJ[3], Adachi R[4], Stevens RL[5], Krilis SA[1]

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Ras guanine nucleotide-releasing protein 4 (RasGRP4) is a mast cell (MC)-restricted guanine nucleotide exchange factor and diacylglycerol (DAG)/phorbol ester receptor. A RasGRP4-defective variant of the human MC line HMC-1 was used to create stable clones expressing green fluorescent protein-labeled RasGRP4 for monitoring the movement of this protein inside MCs after exposure to PMA, and for evaluating the protein's ability to control gene expression. RasGRP4 resided primarily in the cytosol. After exposure to PMA, RasGRP4 quickly translocated to the inner leaflet of the cell's plasma membrane, and subsequently to different intracellular compartments. This was found to be highly dependent on $Phe^{548}$ in the protein's C1 DAG/PMA-binding domain. c-kit/CD117 was loss from

the cell's surface. Interleukin (IL) 13 is pleiotropic immunoregulatory cytokine that plays a prominent role in asthma and other inflammatory disorders. Its effects are mediated via surface receptors that consist of the α subunit of the IL-4 receptor (IL4Rα) linked to the IL-13-specific IL-13Rα1 or IL-13Rα2 subunit. IL-13Rα2 functions in most cell types as a decoy/inhibitory receptor for IL-13-dependent activation of cells. MCs express both types of IL-13 receptors, the mechanism that controls IL-13Rα2 expression to make these cells less responsive to IL-13 than to IL-4 is not known. Transcript-profiling approaches revealed that RasGRP4 profoundly regulated the expression of hundreds of genes in HMC-1 cells. However, the expression of the transcript that encodes the IL-13Rα2 was one of the major genes regulated by the signaling protein, and its transcript increased nearly a 1000-fold in the RasGRP4-expressing HMC-1 cells. A marked increase in the levels of functional IL-13Rα2 protein also was found, and the resulting IL-RasGRP4$^{+}$/IL-13Rα2$^{+}$ cells were poorly responsive to IL-13 as assessed by cytokine induced phosphorylation of STAT6. RasGRP4 regulates signaling pathways that control gene and protein expression in MCs, including the cell's ability to respond to IL-13. The mouse and human RasGRP4 genes resides at sites in their genomes that have been linked to asthma. Thus, dysregulation of RasGRP4 expression in MCs could lead to increased release of mediators when these cells encounter IL-13 in the asthmatic lung due to decreased expression of the inhibitory cytokine receptor.

**5**

**Suppression of normal and malignant Kit signaling by a bispecific antibody linking Kit with CD300a**

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Through its receptor Kit (CD117), stem cell factor (SCF) is the critical regulator of human mast cell differentiation, survival, priming and activation. We previously found that human mast cells express the functional inhibitory receptor CD300a, belonging to the Ig superfamily. The aim of the present work was to assess whether a bispecific antibody linking Kit with CD300a could suppress normal and/or malignant Kit signaling. We therefore synthesized a library of bispecific antibody fragments to examine the effect of linking Kit with CD300a on human cord blood derived mast cells (hCBMC) and on HMC-1 cell line. CD300a exerted a strong inhibitory effect on Kit-mediated SCF-induced signaling, consequently impairing normal mast cell differentiation (cord blood precursors into mature mast cells by tryptase content, survival (annexin V/PI) and activation (tryptase and/or β-hexasaminodone release) *in vitro*. This inhibitory effect was derived from Kit-mediated tyrosine phosphorylation of CD300a and recruitment of the SH2-containing 5' inositol phosphatase 1 (SHIP-1) but not of SH2-containing protein phosphatase 1 (SHP-1). CD300a on the human leukemic HMC-1 cells inhibited the constitutive activation and the subsequent β-hexosaminidose release, but not their survival. Finally, CD300a abrogated the allergic reaction induced by SCF in a murine model of cutaneous anaphylaxis. Our findings highlight CD300a as a novel regulator of Kit in human mast cells, and suggest a role for this receptor also as a suppressor of aberrant Kit signaling in malignant disorders.

**6**

**Induction of mast cell apoptosis – A new way to treat allergy and other mast cell associated diseases?**

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Mast cells are key inflammatory cells not only in allergy but also in many other inflammatory disorders. One possible therapeutic intervention in these diseases could be to induce mast cell apoptosis, thereby reducing the number of tissue mast cells which should lead to less symptoms. To investigate the mechanisms involved in regulating mast cell longevity and survival in health and disease, we have used in vitro developed mast cells, from either gene targeted mice or human cord blood, and animal models. Taking this approach we have delineated the involvement of Bcl-2 family members in the regulation of mast cell survival. We have found that the pro-survival proteins Bcl-2 and Bcl-XL are important for the late phase of mast cell development. A1/Bfl-1 is crucial for activation-induced mast cell survival upon allergic reaction, a phenomenon only observed in connective-tissue like mast cells and not in mucosal-like mast cells. Mast cells deficient in A1 do not survive activation induced by aggregation of FcεRI. Mice deficient in A1 exhibit similar passive cutaneous anaphylaxis (PCA) as wild type mice, demonstrating that A1-deficieny does not affect mast cell reactivity. In contrast, A1-/- mice exhibit less oedema formation and decrease in mast cell numbers in an active cutaneous anaphylaxis (ACA) model. Furthermore, transcriptional regulation of A1 in mast cells is dependent on NFAT, in contrast to lymphocytes where NFκB is the main transcription factor. Of the pro-apoptotic BH3-only proteins, we have identified Puma and Bim to be important for cytokine deprivation-induced mast cell survival, whereas Bax is the predominant executing pro-apoptotic protein in mast cells. Stem cell factor (SCF) regulates mast cell survival by suppressing Bim in two ways; inhibiting its transcription and increasing its proteasomal dependent degradation. By increasing the levels of Bim in mast cells we have been able to induce apoptosis in these cells, even in the presence of SCF or in mast cells with D816V *c-kit* mutations. In summary, we have identified two plausible targets that could be used to induce mast cell apoptosis; inhibition of A1/Bfl-1 for IgE-mediated activation-induced mast cell survival, and induction of Bim in diseases with mast cell hyperplasia.

**7**

**$Ap_4A$ as a second messenger in a novel gene regulation pathway in immunologically activated mast cells.**

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The signaling pathway regulating the intracellular level of diadenosine tetraphosphate ($Ap_4A$) in mast cells, and in other cells derived from the immune system, has not yet been described. We have been studying the regulation of microphthalmia transcription factor (MITF) in mast cells and during our studies we discovered that lysyl-tRNA synthetase (LysRS) forms a complex with MITF. We hypothesized that this association is related to one of LysRS's known "moonlight" functions as a producer of $Ap_4A$. Indeed our studies demonstrated that $Ap_4A$ causes the dissociation of MITF from its inhibitor Hint-1. Here we demonstrate that immunological activation of mast cells by $Fc_{\varepsilon}RI$ aggregation leads to mitogen-activated protein kinase (MAPK) dependent LysRS phosphorylation, resulting in its dissociation from the cytosolic multisynthetase complex (MSC), its translocation to the nucleus and to enhanced $Ap_4A$ production. Furthermore, we show that knock-down of either LysRS or $Ap_4A$ hydrolase modulates $Ap_4A$ accumulation, resulting in changes in the expression of MITF target genes. Thus, we have established $Ap_4A$ as a second messenger in a novel pathway of gene regulation, by exhibiting the presence of enzymatic machinery directly responsible for its synthesis and metabolism in response to external stimulus.

**8**

**Mast cells as modulators of CD8+ T cell responses**

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Mast cells are considered to contribute dominantly to the establishment of an innate immune response. However, mast cells can also act as important participants of the adaptive immune response, by regulating the phenotype and function of the adaptive immunity players (B cells, dendritic cells and T cells). T cells represent a cutting edge for the induction of adaptive immunity and CD8+ T cells have been reported to be recruited *in vivo* by mast cells. Therefore, this study was focused on the crosstalk between mast cells and CD8+ T cells. The potential of mast cells to induce antigen-specific CD8+ immune responses was investigated. We could demonstrate that bone marrow derived mouse mast cells (BMMCs) labelled with OVA-derived OT-I peptide were able to induce activation, cytokine production and proliferation of transgenic, OT-I specific CD8+ T cells. Moreover, blocking of cytokine synthesis by mitomycin C treatment of BMMCs and, to a higher extent, fixation of BMMCs by paraformaldeyde treatment, decreased their ability to specifically activate CD8+ T cells, thus demonstrating that the specific activation of CD8+ by BMMCs is dependent both on membrane-bound peptides and on soluble factors produced by BMMCs. Furthermore, Toll like receptor – specific pre-priming of BMMCs increased their ability to induce antigen specific CD8+ responses. Interestingly, BMMCs were able to induce antigen specific

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proliferation of naïve primary CD8+ T cells *in vivo*. Taken together, these data demonstrate that mast cells can potently induce antigen specific CD8+ responses. This function of mast cells can be modulated by Toll like receptor signals. Thus, it is suggested that mast cells are key regulatory cells at the cross-roads of innate and adaptive immunity.

## 9

### A mite antigen, Der p1, activates dendritic cells (DCs) and stimulates Th2 polarization through a toll-like receptor (TLR)-4 pathway

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**Rationale:** Exposure to mite is implicated in the development and exacerbation of asthma and other allergic diseases. Therefore, we investigated whether a mite allergen, Der p1, directly stimulates DCs and promotes allergic responses.
**Methods:** We cultured mouse bone marrow-derived DCs with Dermatophagoides pteronyssinus (DP) extract or purified natural Der p1. Cell surface expression of co-stimulatory molecules and cytokine production were analyzed by FACS and by ELISA, respectively. Der p1-stimulated DCs were also co-cultured with allogeneic CD4+ T cells, and T cell production of cytokines was analyzed.
**Results:** Both DP extract and purified Der p1 activated DCs, as shown by increased expression of co-stimulatory molecules (CD40, CD80, and CD86) and by IL-6 production. Heat treatment of Der p1 abolished DC expression of co-stimulatory molecules and IL-6 production; proteinase K treatment of Der p1 also abolished IL-6 production. These treatments did not affect the ability of lipopolysaccharide (LPS) to stimulate DCs, suggesting that the effects of Der p1 on DCs are unlikely due to LPS contamination. When stimulated with Der p1 or LPS, DCs from TLR4-deficient mice produced less IL-6 compared to DCs from wild-type mice. Der p1 treatment also downregulated TLR 4 expression in DCs from wild-type mice. When allogenic CD4+ T cells were incubated with Der p1-treated DCs, the T cells showed enhanced production of IL-4, IL-5, and IL-13 and reduced production of IFN-γ. Heat treatment of Der p1 abolished this Th2-driving effect of Der p1.
**Conclusion:** Der p1 activates DCs and facilitates CD4+ T cell production of Th2 cytokines through a TLR4-dependent mechanism. This effect is likely mediated by Der p1 protein itself and not by LPS contamination. Thus, TLR4 may serve as an innate receptor for certain environmental allergens.

## 10

### Role of eosinophils in innate immunity

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Extensive studies of eosinophil functions in parasitic and allergic diseases have underlined their role as effector cells able to release cytotoxic granule proteins, mediating toxicity to helminth parasites but also to normal tissues. However, recent findings, show that eosinophils produce numerous cytokines, chemokines and growth factors, endowing them with a role in normal physiology including immunoregulation.
Eosinophils are of a ancient lineage, with eosinophilic granules, having evolved with helminth parasites for more than 500 million years, suggesting that eosinophil ancestors have participated in innate responses.
Eosinophils express several receptors involved in innate immunity, such as lectin-type receptors (Siglec, mannose receptor), receptors for lipid mediators, complement or chemokine, protease- activated receptors (PAR), whereas mRNA encoding different TLRs have been detected. Taken together, these data suggest that eosinophils represent a new link between innate and adaptive immunity.
In contrast to the numerous studies of eosinophil function in protective immunity against parasitic infections, eosinophil interactions with "danger" signals including tumor cells or pathogens are poorly documented.
In order to analyse the role of eosinophils in innate immunity to bacteria, we have investigated the direct interactions of human eosinophils with mycobacteria. Using *Mycobacterium bovis*- BCG as experimental model, we have evaluated the activating potential of BCG, on eosinophils. Since TLR2 and TLR4 are the main known TLRs involved in interactions of macrophages with different species of mycobacteria, we have analysed their expression by eosinophils, using flow cytometry. TLR2, TLR4 and also TLR7 were detected on eosinophils, with an increased expression under activation. Pre-incubation of eosinophils with blocking antibodies indicate that only TLR2 and not TLR- 4 is involved in eosinophil- BCG interactions, through activation of MAP kinase signalling. These results suggest a role for eosinophils in TLR2-mediated immunity against an intracellular pathogen. As for other granulomatous reactions found around helminth parasites or in chronic inflammatory bowel diseases, these results raise the question of the beneficial or detrimental role of eosinophils.
Together with other studies suggesting a role for eosinophils in viral infections or in tumor immunity, these unexpected findings, opens new conceptual vistas about the role of eosinophils in innate immune responses.

## 11

### Rapid IgE-antigen desensitization of mouse bone marrow derived mast cells inhibits degranulation, STAT6 phosphorylation and calcium release in an antigen-specific manner

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Mouse bone marrow mast cells can be rapidly desensitized to IgE antigens by incremental suboptimal doses but the duration, kinetics, calcium dependency and molecular targets have not been elucidated. In addition, the specificity of desensitizations has not been evaluated.
**Objectives:** We wanted to induce unresponsiveness to optimal doses of DNP-IgE through desensitization and study granule mediator release, signal transduction and response to other antigens such as OVA-IgE.
**Methods:** Mouse bone marrow derived mast cells were sensitized with IgE anti-DNP antibody and desensitized by 12 suboptimal increasing doses of DNP-HSA (optimal dose 1 ng). β-hexosaminidase and calcium release were measured. Desensitized cells were triggered with an optimal dose up to 6 hours after the initial desensitizing dose. To evaluate the specificity of the desensitization, cells were sensitized with IgE anti-OVA and IgE anti-DNP, desensitized to OVA or to DNP-HSA and challenged with DNP-HSA or OVA.
STAT6 and LAT phosphorylation were assessed by Western Blot after desensitization.
**Results:** Desensitized cells release 70-80% less β-hexosaminidase and intracellular calcium when triggered by an optimal dose of DNP-HSA (1ng) as compared to activated cells. Cells remained unresponsive to optimal antigen doses up to 6 hours after being desensitized.
Cells desensitized to DNP-HSA responded to an optimal dose of OVA but not to an optimal dose of DNP-HSA, and cells desensitized to OVA responded to an optimal dose of DNP-HSA but were unresponsive to an optimal dose of OVA.
STAT6 phosphorylation was significantly inhibited after desensitization but LAT phosphorylation was similar in desensitized and optimally activated cells.
**Conclusions:** Rapid IgE-antigen desensitization is an antigen specific process that inhibits β-hexosaminidase and calcium release at optimal antigen doses and can be maintained by the presence of antigen. STAT6 but not LAT phosphorylation is inhibited in the desensitization process.

## 12

### Expression of cysteinyl leukotriene receptors on human cord blood hemopoietic progenitors: Implications for primary prevention of atopy

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**Background:** Allergic disorders involve systemic inflammatory processes, including the effects of cysteinyl leukotrienes (cysLTs) produced by eosinophils, basophils, and other cell types. Two receptor subtypes for cysLTs have been cloned, cysLTR1 and cysLTR2: while mature eosinophils, basophils, monocytes/macrophages, and mast cells express both receptors, blood and marrow CD34+ hemopoietic progenitor cells

have been shown until now to express only cysLTR1. In this novel study, we have examined the expression of cysLTR1 and cysLTR2, and co-expression of IL-5Rα, on human cord blood (CB) CD34+ progenitor cells, in order to assess their potential relevance to the development of atopy.
**Methods:** Non-adherent mononuclear cells (NAMNC) were isolated from human CB by density gradient separation techniques. Flow cytometric staining (FACS) was performed using monoclonal antibodies to CD45, CD34, IL-5Rα, and polyclonal antibodies specific for cysLTR1 and cysLTR2. Data were collected on a six-colour BD LSRII flow cytometer, and analyzed using FlowJo software. FACS-derived cells and methylcellulose colony assays were incubated with optimal and suboptimal concentrations of rhIL-5, GM-CSF, and $LTD_4$. Q-PCR was utilized to evaluate kinetic expression patterns of CB cysLTR1 mRNA in response to IL-5 stimulation.
**Results:** Mean expression on CD34+/CD45+ progenitor cells of cysLTR1 was surprisingly high at 33% (median, 34%; range,19-50%, SD ± 11; n=15); mean expression of cysLTR2 was 41% (median, 42%; range, 13-65%; SD ± 18; n=15). As found previously, IL-5α expression was 3.2% (SD ± 0.6). *Cells co-expressing cysLTR1 with IL-5Rα were 1.8%, and cysLTR2 with IL-5Rα, 1.4% (SD ± 0.2)*. Incubation of CB NAMNCs with IL-5 resulted in an up-regulation of cysLTR1 mRNA, peaking at 48 hours post-stimulation. This kinetic pattern was highly correlated (R=0.993) with GATA-1 transcription factor expression.
**Summary & Conclusions:** CysLTR1 and cysLTR2 are *both* highly expressed on CB CD34+ progenitors, and can be co-expressed with IL-5Rα. While *levels* of expression of the cysLTRs were not modulated by $LTD_4$, stimulation with $LTD_4$ *in vitro* resulted in enhancement of IL-5- or GM-CSF-mediated eosinophil colony formation and differentiation from CB CD34+ cells. These findings suggest a role for cysLTs and their receptors, and interactions with IL-5/IL-5R, in the regulation of hemopoietic progenitor cell differentiation and trafficking in early life, and provide a novel mechanism through which hemopoietic progenitors modulate atopic development.

## 13
### Human eosinophils release glutamate with the potential to modulate survival of a subset of activated T-cells

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**Background:** We have previously shown that human eosinophils constitutively express indoleamine 2,3 dioxygenase (IDO), an enzyme that catabolizes tryptophan, resulting in the generation of various catabolites including kynurenines. The latter targets T-helper cells leading to Th2 bias. The mechanisms by which kynurenines influence T-cell selection remain unknown. Glutamate is an excitatory neurotransmitter in the central nervous system. Prolonged exposure to glutamate leads to neuronal apoptosis through a process called excitotoxicity. Similarly, glutamate-induced excitotoxicity is a major immunoregulatory mechanism on glutamate-receptor-expressing activated lymphocytes found in lymphoid tissue. The expression of glutamate receptors on, and release of glutamate by inflammatory cells is currently unknown. We hypothesize that eosinophils express functional glutamate receptors. We also hypothesized that eosinophils release glutamate with the capacity to modulate T cell function.
**Objectives:** To determine the expression of glutamate receptors/transporters on, and the release of glutamate from human eosinophils. As well, to study the differential expression of glutamate receptors on Th1 versus Th2 cells and the effect of eosinophil-derived glutamate on T cell survival.
**Methods**: RT-PCR and flow cytometry determined the expression of ionotropic (NMDA, AMPA and Kainate) and metabotropic (mGluR1-mGluR8) glutamate receptors. The expression of vesicular and non-vesicular glutamate transporters in human eosinophils and T-cells was examined. Using flow cytometry (Fluo-3AM and Fura Red), we estimated changes in intracellular calcium following activation of eosinophils and T-cells with glutamate and kynurenines. In addition, intracellular cAMP levels were measured following cellular activation with glutamate, using a commercial kit. The release of extracellular glutamate from eosinophils, following adherence to fibronectin or cytokine treatment was measured colorimetrically. Apoptosis in T-cells was determined using annexin-V staining and flow cytometry.
**Results**: Human eosinophils expressed functional mGluR2 and mGluR7, but not NMDA receptors. Freshly isolated eosinophils did not express any of six classes of glutamate transporters. However, adhesion to fibronectin-coated plates rapidly induced the expression of the $Xc^-$ cystine/glutamate antiporter system and led to the release of glutamate within 2hr. In contrast to eosinophils, $CD4^+$ lymphocytes constitutively expressed mGluR5 but only expressed mGluR1 and NMDAR1 following activation with CD3/CD28 beads. Co-culture of glutamate-producing eosinophils with activated $CD4^+$ T-cells enhanced proliferation in a subpopulation of T cells, while promoting apoptosis in others.
**Conclusions:** Eosinophils express functional glutamate receptors. Glutamate released by tissue-dwelling eosinophils may modify T-cell function. Eosinophil-derived products of IDO-mediated tryptophan catabolism, which also act as NMDA receptor agonists in addition to glutamate, may modulate T-cell function.

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## 14
### Amplification of T helper 1 immunity through CD8 T cell to dendritic cell feedback

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Regulatory or suppressor T cells have attracted considerable interest in recent years. One of the first described were CD8 T cells that suppressed IgE. We have investigated the mechanism for this and found that one route through which CD8 T cells inhibit IgE is by stimulating IL-12p70 that promotes Th1 and inhibits Th2 cells. This appears independent of CD8 T cell derived interferon gamma. We have since shown that IL-18 synergizes with IL-12p70 to suppress IgE and promote Th1 cell differentiation. Collectively, these studies suggest that CD8 T cells could induce DCs to produce IL-12p70. However, this has not been formally proven, and the requirements for such an interaction are unknown. To investigate how CD8 T cells induce DCs to produce IL-12p70, we have used an *in vitro* system in which ovalbumin-specific T cell receptor transgenic CD8 T cells and splenic DCs could interact in a peptide specific manner. We showed that although freshly isolated CD8 T cells could induce DCs to up-regulate co-stimulatory molecules (CD80, CD86) and secrete IL-12p40. Induction of bioactive IL-12p70 required CD8 T cells to be pre-activated and an additional signal which could be provided by LPS. We subsequently demonstrated that this effect was peptide-specific and MHC class-I dependent, and that the amount of IL-12p70 induced varied with different CD8 to DC ratios and the amount of LPS used. CD40L:CD40 interactions were shown to play a major part in this process. IL-12p70 production was blocked by anti-CD40L antibodies and little was produced with $CD40^{-/-}$ DCs. CD40L expression on CD8 T cells was increased to over 60% after 3 hours co-culture with peptide pulsed DCs. Comparison of CD8 alpha positive and CD8 alpha negative DC revealed that it was only the CD8 alpha positive DC that produced IL-12p70 although both were able to induce CD8 T cells interferon gamma. Thus activated CD8 T cells can induce CD8 alpha positive DCs to produce IL-12p70 via CD40L and T cell receptor mediated signals.

## 15
### Modulating adaptive immune responses with dendritic cells and innate immune receptors

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The immune system is ignorant or even unresponsive to most foreign proteins that are injected in a soluble, deaggregated form, but when injected together with an adjuvant, these foreign proteins can generate robust immunity and long-lived memory to the antigen. In fact, the nature of the adjuvant is what determines the particular type of immune response that follows, which may be biased towards cytotoxic T-cell responses, antibody responses, particular classes of T-helper responses, or antibody isotypes. Clearly, the ability of a vaccine to skew the response toward a particular type is of paramount importance, because different pathogens require distinct types of protective immunities. Central to this issue

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is a rare but widely distributed network of cells known as dendritic cells (DCs). DCs, which have been called 'Nature's adjuvants,' express pathogen recognition receptors (PRRs), such as the Toll-like receptors (TLRs) and C-type lectins, which enable them to sense and respond to microbes or vaccines. Research in the last decade has demonstrated a fundamental role for DCs in initiating and controlling the quality and strength of the immune response, and emerging evidence suggests an important role for distinct TLRs and C-type lectins in differentially regulating the Th1/Th2/T regulatory balance, by inducing distinct intracellular signaling networks within dendritic cells (DCs). As such, DCs and PRRs represent attractive immune therapeutic targets for allergies, autoimmunities and infections. This presentation will review the emerging themes in the biology DCs and PRRs, with a particular focus on relevance for immune therapy in allergic diseases.

**16**

## Proinflammatory slanDC (6-sulfoLacNAc+ dendritic cells) in atopic dermatitis

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Langerhans cells and inflammatory dendritic epidermal cells are suggested to play important roles in the pathogenesis of atopic dermatitis (AD). We previously described the population of slanDC which have a high T cell stimulatory capacity and which stand out by their high level production of IL-12 as well as TNFα. SlanDC were previously described in human blood as well as in the inflammatory infiltrate of psoriasis and rheumatoid arthritis. In this study we asked whether slanDC may contribute to the immunopathology of atopic dermatitis. With the help a slanDC-specific mAb biopsies obtained from patients with chronic AD lesions (n=15) were studied. SlanDC were frequently found in the perivascular inflammatory infiltrate in all tissues studied. The epidermis was always devoid of slanDC. When studying the phenotype of slanDC in blood (n=16) of patients with AD we observed a significantly higher expression of CD86 (B7-1) compared to healthy controls. SlanDC cultured over night in the absence of any stimuli displayed a strong upregulation of HLA-DR, CD83 and CD86. Again, the expression of CD86 was significantly higher in slanDC from patients with AD. In accordance with their high proinflammatory capacity described previously by our group, LPS-stimulated slanDC of patients with AD displayed a much higher production of TNFα as well as IL-12p40/70 compared to monocytes (p= 0,007 and 0,0001). Furthermore, slanDC of patients with AD when compared with that of healthy controls showed an unaltered capacity to produce high levels of IL-12, as studied on the single cells level. Taken together we demonstrate the presence of proinflammatory slanDC in lesional skin of atopic dermatitis and describe slanDC in blood expressing high levels of the costimulatory molecule CD86. Moreover, slanDC in blood of patients with AD can serve as high level producers of IL-12 as well as TNFα. We therefore conclude that slanDC may significantly contribute to the immunopathology seen in chronic AD.

**17**

## Regulation of Eosinophil Function by Peroxisome Proliferator-Activated Receptor-γ (PPARγ)

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Eosinophils play a pivotal role in the mechanism of allergic diseases. The peroxisome proliferator-activated receptors (PPARs) are a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. To date, three subtypes have been identified, referred to as PPARα, δ, and γ. Among them, PPARγ is not only highly expressed in adipose tissue but also cells involved in the immune system, and it exerts anti-inflammatory activities. The aim of the study was to evaluate the functional roles of PPARγ in human eosinophils. We found that human peripheral blood eosinophils expressed PPARγ at the mRNA and protein levels. Next, to elucidate the role of PPARγ in eosinophil function, we used the pharmacological agonists of PPARγ thiazolidinedions. Stimulation of eosinophils with therapeutic concentrations of a synthetic PPARγ agonists inhibit factor-induced eosinophil activation in terms of chemotaxis, survival, CD69 expression, adhesion, and degranulation. These observations suggest that the use of pharmacological PPARγ agonists may be a novel therapeutic modality for the treatment of allergic diseases including asthma by negatively regulating the eosinophil functions.

**18**

## IgE-mediated Activation of Human Basophils Stimulates Hypoxia-Inducible Factor-1 alpha Accumulation

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**Rationale:** Hypoxia-inducible factor 1 alpha (HIF-1α), the inducible subunit of the HIF-1 transcription complex, facilitates cellular adaptation to low oxygen conditions such as inflammation, tumour growth and ischemic disorders. The protein plays a pivotal role in controlling glycolysis, cell adhesion and angiogenesis. Since allergic responses are associated with increased oxygen consumption and IgE-mediated activation of several kinase cascades that require ATP we asked whether accumulation of HIF-1α protein is induced in human basophils following IgE-dependent stimulation.
**Methods:** Human basophils, which exhibit important effector and pro-allergic immunomodulatory functions, were obtained from buffy coats and purified by magnetic cell sorting. After treatment with or without anti-IgE under varying conditions, basophil reactivities were determined by observing histamine releases and, in parallel, HIF-1α expressions assessed by Western blotting. Additionally, reactive oxygen species generation was measured by luminometric detection and ASK1 activity assessed by a non-radioactive kinase assay following immunoprecipitation.
**Results:** Our data show that HIF-1α protein accumulates in human basophils as early as 10 min following stimulation with anti-IgE and its stabilisation increases further in a time-dependent manner over the next two hours. Furthermore, reactive oxygen species, ASK1 and PI 3-kinase were not involved in anti-IgE-dependent accumulation of HIF-1α protein; although in comparison the PI 3-kinase inhibitor LY294002 (10 μM) substantially blocked IgE-mediated histamine secretion (by more than 80%). Conversely, using the signalling inhibitors PD098059 (10 μM) and SB203580 (1 μM), it was clear that HIF-1α stabilisation is dependent on the MAP-kinases ERK and p38 MAPK.
**Conclusions:** These data clearly demonstrate that IgE-mediated stimulation upregulates HIF-1α stabilization in basophils using signalling pathways that partially differ from those controlling mediator secretions. Moreover, our data strongly indicate a role for HIF-1α protein in sustaining IgE-mediated basophil energy metabolism which protects these cells against programmed death by preventing the depletion of ATP.

**19**

## Pleiotropic roles of formyl-peptide receptors expressed on human basophils

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The formyl peptide receptors (FPRs) are members of the seven-transmembrane, G-protein coupled receptors superfamily, expressed at high levels on polymorphonuclear and mononuclear phagocytes. FPR is the high-affinity receptor for fMLP. Two other homologues of the FPR have been identified, FPR-like 1 (FPRL1) and FPR-like 2 (FPRL2). Because of their capacity to interact with bacterial chemotactic formylated peptides, these receptors are thought to play a role in host defence against microbial infection.
Recently, additional and more complex roles for these receptors have been proposed because FPRL1 and FPRL2 have been found to interact with a variety of pro- and anti-inflammatory ligands associated with different diseases including amyloidosis, Alzheimer, prion disease and HIV. FPRL1 is activated by serum amyloid A, the 42-aa form of β-amyloid ($A\beta_{1-42}$), the prion peptide ($PrP_{106-126}$), lipoxin $A_4$ and the gp41 peptides of HIV-1. Two natural FPRL2 agonists have been characterized: Hp(2-20) produced by *Helicobacter pylori* and urokinase (uPA), a serine protease important in fibrinolysis, tissue remodelling, tumor invasion and also required for leukocyte migration.

Mast cells and basophils are the main effector cells in IgE-mediated allergic responses, but they also play important roles in innate immune responses against bacteria by releasing proinflammatory mediators and cytokines.
We have demonstrated that human basophils express all the three receptors for fMLP, important for chemotaxis and the release of proinflammatory mediators. In particular, we provide the first evidence that two HIV-1 gp41 peptides are potent chemoattractants for human basophils through the interaction with FPRL1. This suggests that FcepsilonRI$^+$ cells can contribute to the dysregulation of the immune system in HIV-1 infection. We have also demonstrated that basophils infiltrate the gastric mucosa of patients with chronic gastritis caused by *H. pylori* and that Hp(2–20) is a potent chemoattractant for basophils through interaction with FPRL1 and FPRL2. Finally, uPA and uPAR are potent chemoattractants for basophils through the engagement of FPRL1 and FPRL2.
These new findings have greatly expanded the role of FcepsilonRI$^+$ cells and call for more in-depth investigation of the role of basophils and mast cells in several pathophysiological conditions.

## 20
### Are basophils present in skin urticaria lesions?

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**Background:** In spite of the prevalence and morbidity of chronic urticaria, we are only beginning to understand its physiopathology, we do not have a curative treatment and many questions remain unanswered on the physiopathology of this disease. Most studies have focused on the properties of chronic urticaria sera. We have previously demonstrated that basophil of CU patients have special releasability features. It has also been demonstrated basopenia in a number of patients with CU.
**Material and Methods:** We performed immunohistochemical analysis of 16 skin biopsies of chronic urticaria using specific basophil antibodies 2D7 and BB1 and c-kit. We compared the result with cell line KU812F and purified peripheral blood basophils from a normal donor. In addition co-immunostaining technique with BB1 and C-kit was done. We also studied CD63 expression upon stimulation with anti-IgE in peripheral blood basophils of patients suffering from CU.
**Results:** When CD63 expression was studied, the number of basophils was very low and no CD63 expression was induced upon anti-IgE stimulus. Chronic urticaria biopsies showed positive granular immunostaining against 2D7, BB1 and c-kit. Peripheral blood basophils showed intense immunostaining against 2D7, BB1 and C-Kit. The majority of KU812F cells showed intense c-Kit immunoreactivity with a cytoplasmic and granular pattern. However, most KU812F cells showed no immunoreactivity against 2D7 or BB1.
**Conclusion:** Based on our results it seems that basophils are present in skin CU lesions. It is tempting to speculate that the reason of the low basophil number could be due to the skin recruitment of these cells. Our data also support prior reports indicating that basophils of patients have a diminished response to anti-IgE.

## 21
### Allergic Inflammation: A mast cell - basophil affair?

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Current views of the pathogenesis of immediate-type hypersensitivity diseases emphasize the role of mast cells in the immediate response to allergen, while APC's, Th2-lymphocytes and eosinophils are regarded as the major players in late-phase reactions and chronic allergic inflammation. Here we propose that human allergic inflammation is predominantly a mast cell – basophil affair which is triggered by allergen specific IgE followed by attraction and activation of basophils. Indeed, together - and in synergy - they produce high levels of all the mediators and cytokines which have been implicated in allergic inflammation (e.g. histamine, PGD2, LTC4, GM-CSF, IL-3, IL-4, IL-5, IL-13, NGF, various chemokines). The major regulators of this co-operation are basophil-derived IL-4 and mast cell-derived IL-3, although we found that other still unknown product(s) of activated basophils can further augment Th2-type cytokine generation. Using genomic and proteomic approaches we also identified novel potential mediators of allergy which are formed when blood basophils are exposed to human mast cells activated by IgE-receptor crosslinking: Granzyme B, a protease relatively resistant to anti-proteases of bodily fluids which is released in the asthmatic late phase reaction; and the vitamine A metabolite, retinoic acid, which can regulate the function of myeloid, lymphoid and resident cells. In contrast to neutrophil and eosinophil granulocytes, basophils are long-lived cells due to constitutively high levels of anti-apoptotic proteins, further indicating an important immunoregulatory role of this rare cell type. This efficient spontaneous survival is regulated by a distinct set of only a few ligands. Basophil apoptosis is also efficiently prevented by mast cell-derived factors, indicating that basophils persist for prolonged time at sites of mast cell activation. Finally, the co-operation of mast cells and basophils can lead to synergistic networks for novel - mainly tissue-derived- cytokines, which recently emerged as important mediators of Th2-type inflammatory conditions (TSLP, IL-25, IL-33). We thus propose that basophils and mast cells co-operatively play a central pro-inflammatory and immunoregulatory role in chronic allergic inflammation by integrating antigen-dependent (allergen +specific IgE) and antigen-independent (tissue-derived) inputs. These circuits eventually lead to an increasingly antigen-independent pathology in chronic allergic disease.

## 22
### Retinaldehyde dehydrogenase-II induction and retinoic acid formation in human basophils interacting with IgE-activated mast cells

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Retinoic acid (RA), the active metabolite of vitamin A (retinol), is an important autocrine and paracrine mediator that regulates the transcriptional activation or repression of numerous genes in almost any cell type by activating nuclear receptors (RAR/RXR heterodimers). The rate-limiting step in RA formation from retinol in vivo is at the level of dehydrogenation of retinal by retinaldehyde dehydrogenases. Retinaldehyde dehydrogenase-II (RALDH2) is the most efficient and selective enzyme creating localized RA gradients needed for proper embryonic development. However, despite extensive literature about pharmacological effects of RA and increasing evidence for a role of RA in immune functions, very little is known about the control of local RA synthesis in adults. Using a human ex-vivo model of allergic inflammation by co-incubating IgE-receptor-activated mast cells with blood basophils, we observed prominent induction of a protein which was identified as RALDH2 by mass spectroscopy. RALDH2 was selectively induced in basophils by mast cell-derived IL-3 involving PI-3-kinase and NF-kappaB pathways. Among a large number of proinflammatory and immunoregulatory cytokines studied, IL-3 was the only effective inducer of RALDH2. Importantly, neither constitutive nor inducible RALDH2 expression was detectable in any other human myeloid or lymphoid leukocyte type, including plasmacytoid dendritic cells expressing IL-3-receptors at a similarly high density as blood basophils. RA generated by RALDH2 in basophils modulates IL-3-induced gene expression in an autocrine manner, providing positive (CD25) as well as negative (granzyme B) regulation. In addition, co-culture with naïve T cells revealed a paracrine, immunoregulatory role of basophil-derived RA by promoting Th2-polarization and inducing the mucosal-homing integrins alpha 4 ($\alpha$4) and beta 7 ($\beta$7). Thus, RA must be viewed as a novel basophil mediator with a high potential of regulating diverse functions of immune and resident cells in allergic inflammation and other Th2-type immune responses. Our data will also be discussed in the context of recent findings in the mouse indicating a role of RA in immune-homeostasis of the gut-associated lymphoid tissue.

# Abstracts

## 23

### Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors

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Chemokine receptors regulate the trafficking of leukocytes by mediating chemotaxis and by their influence on the expression and/or affinity of leukocyte integrins. Using blocking mAb, we previously showed that antigen-induced recruitment of MCp to the lung requires interaction of a4 integrins on the MCp with endothelial VCAM-1. In seeking a chemokine component we found that CXCR2 deficient but not CCR3 or CCR5 deficient sensitized and antigen-challenged mice have significantly fewer lung MCp one day post-challenge and fewer tracheal intraepithelial MC one week post-challenge, implying that recruited MCp provide the source for these mature MC. Unexpectedly, reconstitution of sensitized, sublethally irradiated +/+ and -/- mice with bone marrow cells of either genotype indicated that expression of CXCR2 by the migrating MCp was not required. Instead, receptor function by resident lung cells was required since normal BM did not reconstitute MCp recruitment in irradiated CXCR2-/- mice. The reduced MCp influx into the lung of CXCR2-/- mice was accompanied by reduced induction of VCAM-1 transcripts and reduced endothelial surface expression. Thus, these studies demonstrate a novel role for a chemokine receptor in regulating endothelial VCAM-1 expression, MCp migration and the level of intraepithelial mast cells in the lung of aerosolized antigen challenged mice.

## 24

### Fibroblasts stimulated by mast cells produce IL-6 that vice versa supports mast cell survival

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**Rationale**: Human intestinal mast cells (MC) are key effector cells in allergic reactions but also involved in host defense and tissue remodeling processes such as wound healing, angiogenesis, and fibrogenesis. We have shown previously that human intestinal fibroblasts (FB) suppress apoptosis in human intestinal MC independent of the mast cell growth factor stem cell factor (SCF), IL-3, or IL-4 but the implicated factor remained elusive. Here, we identified this factor as IL-6. **Methods**: Human intestinal MC and FB were isolated from surgical tissue specimens using a four-step enzymatic dispersion method. Following overnight culture, MC were separated from adherent FB and purified using MACS-technique. **Results** We found that intestinal FB are capable of producing IL-6 provided that they were stimulated directly by MC in co-culture or by MC mediators such as TNF-α, IL-1β, tryptase, or histamine. In order to investigate the role of IL-6 for the survival of intestinal MC, MC were incubated with different concentrations of IL-6. Low nanogramm amounts of IL-6 supported MC survival up to 2 weeks in culture. In contrast to treatment with SCF, all MC incubated with IL-6 died after 3 weeks of culture. Interestingly, culture of MC with supernatants of FB stimulated with TNF-α or IL-1β had similar effects as IL-6. MC survived for 2 weeks in culture, but not longer. Furthermore, MC survival in culture with FB supernatant could be blocked using an anti-IL-6 Ab. **Conclusion:** Taken together, these findings suggest that MC factors trigger FB to produce IL-6, and, vice versa, that IL-6 supports MC survival.

## 25

### New genetic mouse models for the investigation of mast cell function in vivo

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While best known as effector cells in type I allergy, mast cells are also key players in host defence against pathogens. In addition, mast cells were implicated in tissue remodeling, wound healing and transplant tolerance. Investigation of this important cell type, however, was severely hampered by the scarcity of mast cells in the tissues. Until today, *in vivo* analysis of mast cell-specific functions of individual genes relied on the reconstitution of genetically mast cell-deficient mice with mast cells differentiated *in vitro* from bone marrow of mice deficient for the gene of interest. Herein, we use the Cre/loxP recombination system for conditional mast cell-specific mutagenesis *in vivo*. In a bacterial artificial chromosome (BAC) transgene, we expressed a Cre-cassette under the control of the mast cell protease 5 (Mcpt5) promoter. Mcpt5-Cre mice showed highly efficient Cre-mediated recombination in mast cells, but not in other cell types and are presently used for mast cell-specific inactivation of various genes. The cross of the new transgenic line to mice expressing a human diphtheria toxin receptor upon Cre-mediated deletion of a loxP-flanked stop element ("iDTR" mice), allows inducible ablation of the mast cell lineage *in vivo*. In a pilot experiment, the population of peritoneal mast cells was no longer detectable 24 hours after a single i.p. injection of diphtheria toxin, indicating efficient lineage ablation. In addition, we have generated mice carrying a modified transgene encoding a Cre-estrogen receptor (ERT2)-fusion protein allowing *in vivo* induction of Cre-mediated gene"knock out" by administration of tamoxifen. The new Cre-transgenic mouse lines will be useful tools for the investigation of mast cell biology.

## 26

### Virally activated human mast cells induce the selective chemotaxis of NK cells via a CXCL8 dependent mechanism

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Mast cells are found at many sites of potential infection such as the skin and airways mucosa. They recognize invading pathogens and produce mediators that promote a vigorous and effective immune response. Mast cells can induce the migration of multiple cell types in allergic disease and bacterial infection, but their ability to recruit anti-viral effector cells, such as natural killer (NK) cells and T cells has not been fully elucidated. To investigate the role of human mast cells in response to virus associated stimuli, human cord blood-derived mast cells were stimulated with polyinosinic·polycytidylic acid poly (I:C), a double-stranded RNA analogue, or infected with the double-stranded RNA reovirus serotype 3 Dearing for 24 hours. Mast cells responded to stimulation with Poly (I:C) by producing a distinct chemokine profile, including CCL4, CXCL8, and CXCL10, in the absence of significant degranulation. Reovirus infection of cord blood-derived mast cells was confirmed by flow cytometry, such infection of mast cells also induced substantial CXCL8 production. Supernatants from both reovirus and Poly (I:C) activated mast cells induced substantial NK cell chemotaxis that was highly dependent on CXCL8 and its receptor, CXCR1 expressed on NK cells. However, NK cell chemotaxis was independent of CXCR3 and CXCR4. These results suggest a novel role for mast cell derived CXCL8 in the recruitment of human NK cells to sites of viral infection.

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## 27

### TRPC5/Orai1/STIM1-dependent Store-operated Entry of $Ca^{2+}$ Regulates Degranulation in a Mast Cell Line

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Degranulation of mast cells in response to antigen (Ag) or calcium mobilizing agents such as thapsigargin, is dependent on depletion of intracellular stores of $Ca^{2+}$ and the ensuing influx of external $Ca^{2+}$, a

process referred to as store-operated calcium entry (SOCE). $Ca^{2+}$-entry was initially thought to occur through a calcium-release activated calcium current ($I_{CRAC}$ or CRAC) that was first identified in mast cells by patch-clamp techniques and recently shown to require Orai1, a plasma membrane channel protein, and STIM1, a $Ca^{2+}$-sensor in the endoplasmic reticulum. CRAC is a $Ca^{2+}$-selective current that is dependent on external $Ca^{2+}$ to maintain maximal conductance. Replacement of $Ca^{2+}$ with either $Sr^{2+}$ or $Ba^{2+}$ diminishes CRAC activity. The conundrum is that earlier studies clearly demonstrated that stimulated mast cells become highly permeable to a variety of divalent cations including $Sr^{2+}$ and that such ions can support degranulation in the absence of $Ca^{2+}$. We have investigated the role of transient receptor potential canonical (TRPC) channels as potential mediators of $Ca^{2+}$- and $Sr^{2+}$- entry in mast cells as some TRPCs are known to be activated by store depletion, to conduct divalent cations such $Sr^{2+}$ and $Ba^{2+}$ in addition to $Ca^{2+}$, and to associate with Orai1.

Single-cell imaging studies were conducted in the RBL-2H3 mast cell line following overexpression or knock down of Orai1, STIM1, or individual TRPCs and permutations thereof. We found that, among the various TRPCs that are expressed in RBL-2H3 cells, TRPC5 was essential for influx of $Sr^{2+}$, optimal influx of $Ca^{2+}$, and degranulation in the presence of either $Sr^{2+}$ or $Ca^{2+}$. The key observations were these. Overexpression of STIM1 and Orai1 allowed entry of $Ca^{2+}$ but not $Sr^{2+}$ while overexpression of TRPC5 allowed entry of both $Ca^{2+}$ and $Sr^{2+}$. However, optimal influx of $Ca^{2+}$, entry of $Sr^{2+}$, and degranulation required the combination of TRPC5, STIM1, and Orai1 as demonstrated by knock down of each of these proteins by inhibitory RNAs. These and other observations suggest that the $Sr^{2+}$-permeable TRPC5 couples with STIM1 and Orai1 in a stoichiometric manner to enhance entry of $Ca^{2+}$ to generate a necessary signal for degranulation.

## 28
### SNARE proteins in human mast cell degranulation: Role of SNAP23, Syntaxin-4, VAMP-7, and VAMP-8

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**Rationale:** Mediator release of mast cells is a key process in allergic reactions. The events facilitating the fusion of granule and plasma membrane in the course of mast cell degranulation are not clear. SNARE (Soluble NSF Attachment Protein Receptors) proteins have been demonstrated to be involved in fusion of opposing membrane layers during exocytosis. Here, we analyzed expression of SNARE isoforms in human mast cells and which of them might be crucial for mast cell degranulation. **Methods:** Human mast cells were isolated and purified from surgical specimen of intestinal mucosa, using enzymatic digestion and MACS-technique. SNARE-protein expression was demonstrated employing RT-PCR and Western blot. Interaction of SNAREs was analyzed by immunofluorescence and immunoprecipitation. Mediator release and translocation of SNARE proteins was analyzed after stimulation of the cells using $10^{-6}$ M iono/PMA or IgE and anti-IgE antibody, respectively. **Results**: Mature primary human mast cells express the tSNAREs (=target SNAREs) Stx-1B, Stx-2, Stx-3, Stx-4 and SNAP-23, but not SNAP-25, and the vSNAREs (=vesicular SNAREs) VAMP-3, VAMP-7, and VAMP-8. VAMP-2, which has been demonstrated to play a key role in eosinophil exocytosis, was only expressed at very low levels. SNAP-23 formed complexes with VAMP-7 and Syntaxin-4. Fluorescence microscopy revealed translocation of both VAMP-7 and VAMP-8 to the plasma membrane upon stimulation of the cells using $10^{-6}$ M iono/PMA. Furthermore, inhibition of SNAP-23, Syntaxin-4, VAMP-7 and VAMP-8 in streptolysin-O permeabilized mast cells significantly decreased mediator release. **Conclusion**: Human mast cells express a specific pattern of SNARE isoforms which are able to form stable complexes. SNAREs might play a major role in human mast cell exocytosis; inhibition of which could represent a novel therapeutical approach in treatment of allergic disorders.

## 29
### Activation of Protein Kinase D1 in Mast Cells in Response to Innate, Adaptive, and Growth Factor Signals

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Little is known about the function of the serine/threonine protein kinase D1 (PKD1) in mast cells. We sought to identify ligands that activate PKD1 in mast cells and to address the contributions of this enzyme to mast cell activation. Mouse bone marrow-derived mast cells (BMMC) contained both PKD1 mRNA and immunoreactive PKD1 protein. Activation of BMMC through TLR2, Kit, or FcεRI with palmitoyl-3-cysteine-serine-lysine-4 (Pam3CSK4), stem cell factor (SCF), or crosslinked IgE, respectively, induced activation of PKD1, as determined by immunochemical detection of autophosphorylation. Activation of PKD1 was inhibited by the PKD1/PKC inhibitor Gö 6976 but not by broad-spectrum PKC inhibitors, including bisindolylmaleimide (Bim) I. Pam3CSK4 and SCF also induced phosphorylation of heat shock protein 27, a known substrate of PKD1, and the phosphorylation was inhibited by Gö 6976 but not Bim I in BMMC. This pattern also extended to activation-induced increases in mRNA encoding the chemokine CCL2/MCP-1 and release of the protein. In contrast, both pharmacologic agents inhibited exocytosis of β-hexosaminidase induced by SCF or crosslinked IgE. Our findings establish that stimuli representing innate, adaptive, and growth factor pathways activate PKD1 in mast cells. In contrast with certain other cell types, activation of PKD1 in BMMC is largely independent of PKC activation. Furthermore, our findings suggest that PKD1 preferentially influences transcription-dependent production of CCL2, whereas PKCs predominantly regulate the rapid exocytosis of preformed secretory granule mediators.

## 30
### The role of PI3K for the function of gastrointestinal mast cells

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Phosphoinositide 3-kinases (PI3Ks) are important for diverse physiological reactions. We have previously shown that mice lacking the p85α regulatory subunit of class IA PI3K ($PI3K^{-/-}$ mice) lack gastrointestinal mast cells and are more susceptible to the infection by intestinal nematode *Strongyloides venezuelensis* than wild type mice. The purpose of this study is to understand the role of PI3K in the development of gastrointestinal mast cells in a steady state and that in mastocytosis upon nematode infection. Limiting dilution analysis showed that the frequency of mast cell precursors in the intestine of $PI3K^{-/-}$ mice was greatly reduced compared to wild type mice, suggesting that either the migration or proliferation of precursors is impaired. The c-$Kit^{+}\alpha4\beta7^{+}$ population in the bone marrow contained a high number of precursors. IL-3-induced c-$Kit^{+}\alpha4\beta7^{+}$ BMMCs from $PI3K^{-/-}$ mice were impaired for the migration to the intestine compared to those from wild type mice. The *in vivo* proliferation of $PI3K^{-/-}$ c-$Kit^{+}\alpha4\beta7^{+}$ BMMCs in the intestine after transfer and their *in vitro* proliferation in response to SCF were significantly impaired. Furthermore, SCF-induced haptotaxis of c-$Kit^{+}\alpha4\beta7^{+}$ BMMCs on MAdCAM1-coated surface was impaired in the absence of PI3K. These results show that the migration of precursor cells to the intestine and the proliferation of precursor cells are impaired in the absence of PI3K in a steady state condition. It has been reported that IL-3 plays an important role in nematode-induced mastocytosis. PI3K/IL-3 double deficient mice were significantly more sensitive to the infection by *S. venezuelensis* than each single deficient mouse line as mastocytosis in the intestine and the induction of serum mMCP1 were severely impaired in double deficient mice. Mastocytosis and the induction of serum mMCP1 were also defective in a food allergy induction model, indicating the critical role of IL-3 in the induction of mastocytosis. Finally, transfer of wild type but not IL-3-deficient BMMCs restored mastocytosis, indicating that IL-3 acts in an autocrine manner on mucosal mast cell precursors to support differentiation of mature mucosal mast cells during intestinal inflammation. Our results collectively indicate that PI3K and IL-3-mediated signaling differentially regulate mast cell differentiation in the gastrointestinal tract.

# Abstracts

## 31
### Expression and release of secretory phospholipases $A_2$ from human lung mast cells

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Secretory phospholipases $A_2$ ($sPLA_2$s) are enzymes involved in the biosynthesis of proinflammatory lipid mediators. Different $sPLA_2$ isoforms are released from inflammatory cells and can be retrieved in plasma and biological fluids from patients with inflammatory and allergic disorders. Mast cells play a crucial role in the pathogenesis of allergic disorders such as rhinitis and bronchial asthma. In initial experiments we evaluated the levels of $sPLA_2$ activity in the bronchoalveolar lavage fluid (BALF) of patients with bronchial asthma. Activity of $sPLA_2$ was determined as the hydrolysis of $^3$H-oleic acid ($^3$H-OA) from radiolabeled *E. Coli* membranes and expressed as pmoles of $^3$H-OA hydrolyzed/ml/min. $PLA_2$ activity was significantly higher ($p<0.01$) in the BALF of asthmatics (28.3±2.2; n=14) as compared to that of the control group (9.3±0.9; n=19). The $PLA_2$ activity in the BALF of asthmatics was blocked by dithiothreitol (DTT: 10 mM) and Me-Indoxam (10 μM), but not by phenylmethylsulfonyl fluoride (PMSF: 2 mM) indicating that it was due to a secretory (not cytosolic) form of $PLA_2$. These results indicate that a specific $sPLA_2$ activity is detectable in the human airways and is increased in patients with bronchial asthma. In another group of experiments we examined whether lung mast cells were a source of the $sPLA_2$ activity released in the airways of asthmatics. Incubation (15-120 min, 37°C) of purified (>95%) human lung mast cells (HLMC) with anti-IgE (1 μg/ml) induced the release of $sPLA_2$ with a time-course comparable to that of the release of histamine. A significant correlation ($r = 0.732$, $p<0.05$) was found between maximal release of $sPLA_2$ and histamine. Activity of $sPLA_2$ released by anti-IgE-activated HLMC was blocked by DTT (10 mM), Me-Indoxam (10 μM) and LY311727 (10 μM), but not by AZ-1 (10 μM) and PMSF (2 mM). These pharmacological characteristics suggest that HLMC synthesize at least two isoforms (hGIIA and hGIIE) of $sPLA_2$. RT-PCR experiments confirmed that HLMC express mRNAs for these two isoforms of $sPLA_2$. These data indicate that mast cells can be an important source of $sPLA_2$s in the airways of patients with bronchial asthma.

## 32
### The urokinase/urokinase receptor: A novel target for the treatment of allergic diseases

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Mast cell and basophils express the tetrameric high affinity receptor for IgE (FcεRI). Human FcεRI cells are considered primary effectors cells of allergic disorders. Basophils circulate in the blood and are able to migrate into tissue at sites of inflammation. The mast cell is a tissue-based immune cell of bone marrow origin. The urokinase-mediated plasminogen activation (PA) system is involved in many physiological and pathological events that include cell migration and tissue remodelling, such as embryogenesis, ovulation, inflammation, wound healing, angiogenesis, and tumor invasion and metastasis. Urokinase plasminogen activator (uPA) binds a specific high affinity surface receptor (uPAR). The uPA-uPAR system is crucial for cell adhesion and migration, and tissue repair. We have investigated the presence and function of the uPA-uPAR system in human basophils and mast cells. The expression of uPAR was found at both mRNA and protein levels. The receptor was expressed on the cell surface of basophils and mast cells, in the intact and cleaved forms. The cells did not express uPA at either the protein or mRNA level. Inactivation of uPA enzymatic activity by di-isopropyl fluorophosphates did not affect its chemotactic activity. A polyclonal antibody against uPAR inhibited uPA-dependent basophil chemotaxis. The uPAR-derived peptide 84-95 ($uPAR_{84-95}$) induced basophils chemotaxis. Basophils expressed mRNA for the formyl peptide receptors: formyl peptide receptor (FPR), FPR-like 1 (FPRL1) and FPRL2. The FPR antagonist cyclosporine H prevented chemotaxis induced by FMLP, but not that induced by uPA and $uPAR_{84-95}$. uPA is a potent chemoattractant for basophils acting through the exposure of the chemotactic epitope $uPAR_{84-95}$, that is an endogenous ligand for FPRL2 and FPRL1.

## 33
### Nitration Of Aldolase: A Critical Post-Translational Modification In Nitric Oxide-Mediated Regulation Of Mast Cell Function?

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Nitric oxide (NO) is a short-lived free radical that plays a critical role in the regulation of cellular signalling. Mast cell (MC) derived NO as well as exogenous NO regulates MC activities. At a molecular level NO acts to modify protein structure and function through several mechanisms including protein tyrosine nitration. We hypothesized that protein tyrosine nitration, a post-translational modification mediated by peroxynitrite, a by-product of NO metabolism plays a regulatory role in MC. Using a hypothesis-generating proteomic approach, we identified an enzyme in the glycolytic pathway, aldolase, as a target for nitration in MC. This is the first report of tyrosine nitration in a human MC line (HMC-1). Nitrated proteins of HMC-1 were assessed using two-dimensional electrophoresis and western blot with anti-nitrotyrosine antibody. In normally cultured HMC-1 there were many immunoreactive spots detected by anti-nitrotyrosine antibody, confirming the existence of constitutive protein tyrosine nitration in this MC. Mass spectrometry was used to characterize proteins selectively nitrated upon treating the cells with S-Nitrosoglutathione (SNOG), a NO donor. Treatment with 500μM of SNOG for 4 hr selectively nitrated tyrosine residues at position 3 and 59 of aldolase A in HMC-1 cells. This nitration was associated with reduced aldolase enzymatic activity and intracellular increase in its substrate, fructose 1,6 bisphosphate (FBP). Since FBP has been reported to inhibit MC degranulation, we studied its effect on MC function. Exogenous FBP treatment resulted in a dose-dependent reduction of adenosine receptor-mediated IL-8 production by HMC-1. Preliminary experiments with LAD-2, another human MC line and human cord blood derived MC also revealed aldolase nitration upon treatment with SNOG. Thus aldolase appears to be a target in NO-mediated control of function in several types of MC. Aldolase nitration has the potential to regulate MC function through multiple mechanisms including elevated FBP levels. In other cell types FBP can influence enzymes like phospholipase Cγ (PLCγ) and phospholipase D2 (PLD-2), or the intracellular messenger inositol triphosphate (IP3). Analyses of the possible links between aldolase nitration, altered FBP levels and the regulation of MC function may help identify novel therapeutic targets to treat diseases.

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## 34
### Mast cells as a source of 15-lipoxygenase products

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Mast cells play a key role in the pathophysiology of allergy and asthma. These cells exert their effector functions by releasing a variety of proinflammatory and immunoregulatory compounds. 15-Lipoxygenase type-1 (15-LO-1) is a lipid peroxidizing enzyme leading to formation of pro- and anti-inflammatory mediators in the arachidonic acid cascade. The enzyme has previously been shown to be expressed in airway epithelial cells, eosinophils, reticulocytes and monocytes /macrophages. The primary 15-lipoxygenase metabolite of arachidonic acid, 15-hydroxyeicosatetraenoic acid (15-HETE), was suggested to be involved in bronchial asthma with increased formation in lung specimens from patients with asthma as compared to non-asthmatics. Here we report that interleukin-4 (IL-4) induced the expression of 15-LO-1 in human mast cells (derived from stem cells in human umbilical cord blood, cord blood derived mast cells; CBMC) as demonstrated by RT-PCR, western blot and immunocytochemistry. The major metabolite of arachidonic acid formed via the 15-LO pathway in IL-4 treated CBMC was identified as 15-ketoeicosatetraenoic acid (15-KETE, also named 15-oxo-ETE) with smaller amounts of 15-HETE as identified by HPLC and mass spectrometry (MS/MS). Furthermore, immunohistochemical stainings demonstrated the expression of 15-LO-1 in mast cells in lung and skin in vivo. Activation of the 15-LO-1 pathway in CBMC was shown with arachidonic acid, osmotic activation with mannitol as well as immunological challenge with anti-

IgE. In conclusion, the expression of 15-LO-1 and release of 15-LO-1 derived products by mast cells may contribute to the role of these cells in allergy, asthma and other inflammatory diseases.

**35**

**Evidence that mast cell degradation of neurotensin contributes to survival in a mouse model of sepsis**

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Sepsis is a complex, incompletely understood and often fatal disorder, typically accompanied by hypotension that is considered to represent a dysregulated host response to infection. Neurotensin (NT) is a 13-amino-acid peptide that, among its multiple effects, induces hypotension. We found that intraperitoneal and plasma concentrations of NT were increased in mice after caecal ligation and puncture (CLP), a model of sepsis, especially during severe CLP associated with a high mortality. In a model of moderate CLP, levels of NT at 24-28 h after CLP were significantly higher in genetically mast cell-deficient WBB6F1-*Kit*$^{W/W\text{-}v}$ mice than in ether the corresponding wild type mice or in WBB6F1-*Kit*$^{W/W\text{-}v}$ mice that had been engrafted i.p. with wild type bone marrow-derived cultured mast cells. Mice treated with a pharmacological antagonist of NT, NT-deficient mice or neurotensin receptor 1 (Ntsr1)-deficient mice exhibited reduced mortality after severe CLP. In mice, mast cells reduced levels of NT after the i.p. injection of the peptide, and reduced NT-induced hypotension and CLP-associated mortality, and optimal expression of these effects required mast cell expression of Ntsr1. Mouse mast cells expressed the NT-degrading enzyme, neurolysin (NLN), and the active enzyme was detected in both cytosol and membrane fractions of these cells. By using a shRNA based approach, we obtained evidence that NLN is more important than mast cell-associated carboxypeptidase A (mMC-CPA) in reducing the hypotension induced by the i.p. injection of the mice with NT. Moreover, we obtained *in vitro* and *in vivo* evidence that mouse peritoneal mast cells can degrade NT without undergoing extensive degranulation. These findings show that NT can contribute to sepsis-related mortality in mice, particularly in a severe model of CLP that is associated with high mortality, and that mast cells can regulate NT concentrations during sepsis or after injection of NT *in vivo*. These observations strongly suggest that mast cell-dependent reduction in NT levels contributes to the ability of mast cells to enhance survival after CLP in mice. In a pilot study of patients admitted to the intensive care unit, we found that plasma concentrations of NT were elevated to levels similar to those in mice with severe CLP (median: 224 fmol/ml [range: 65-1715 fmol/ml, $n = 17$], versus a median of 30 fmol/ml [range: 13.8-180 fmol/ml, $n = 14$] in healthy controls, $P < 0.0001$). Notably, plasma concentrations of NT were similarly elevated in subjects with cardiogenic shock (median: 309 fmol/ml [range: 10-2176 fmol/ml, $n = 6$], $P < 0.04$ vs. values for healthy control subjects). Taken together with our findings in mice, these human data raise the possibility that NT might contribute to hypotension in patients with either sepsis or cardiogenic shock, and therefore that inhibiting pathological actions of NT may confer benefit in these settings.

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**Gene expression profiling after acute versus chronic allergen airway exposure reveals distinct gene clusters**

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**Background:** Asthma is a chronic inflammatory disease characterized by increased mucus production, development of airway hyperreactivity (AHR) and airway remodeling. The latter is associated with chronic inflammation, increased smooth muscle mass, goblet cell hyperplasia and subepithelial fibrosis. Up to date, little is known about the genetic and molecular mechanisms underlying this process.
**Aim:** To compare gene expression profiles after acute versus chronic allergen airway exposure in a mouse model of allergic airway disease.
**Methods:** Two different mouse models were used: For the acute model, Balb/c mice were sensitized by intraperitoneal injection of Ovalbumin/Alum (OVA) and challenged twice via the airways with allergen after four weeks. For the chronic model, sensitized mice underwent repetitive allergen airway challenges for eight or twelve weeks, respectively.
Remodeling was assessed by measurements of inflammatory cells and cytokine levels in bronchoalveolar lavage (BAL) fluid, *in vivo* lung function, immunoglobulin serum levels, and collagen content in lung tissues. Gene expression profiles were analyzed from lung tissues, spleen, lymph nodes and ganglia.
**Results:** Development of AHR was greatest after acute airway challenges, whereas highest numbers of inflammatory cells were detected after 8 weeks. Similar, serum IgE concentrations were raised after 8 and 12 weeks of chronic challenges compared to the acute model.
Significant gene regulation between OVA-treated and control mice was detected in lung tissues and lymph nodes. Corresponding to the remodeling process, we were able to identify specific gene expression patterns with genes of the integrin signal pathway, apoptosis signal pathway, angiogenesis and B-cell activation significantly regulated in the chronic asthma model. Single genes such as protease inhibitors and lipocalin were selected and further analyzed by QPCR, western blotting, immunohistology and blocking or knock-out experiments for validation of biological function.
**Conclusions:** The micro array technology provides a powerful tool to study gene expression in complex diseases such as asthma. We confirmed many genes already known to be relevant to asthma, but also detected a great variety of novel target genes and pathways involved in acute and chronic asthma pathogenesis, which may be relevant for future diagnostic or therapeutic approaches.

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**Ichthyosis prematurity syndrome: Monogenetic skin disease with concomitant hallmarks of allergy**

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Allergic diseases are complex genetic disorders with heterogeneous phenotype, largely attributed to the interactions among many genes and between these genes and the environment. Hallmarks of allergic diseases are elevated serum immunoglobulin E (IgE) and increased numbers of eosinophils: both are thought to be central to the pathology of the disease process. An alternative approach to identify basic mechanisms of multifactorial disorders such as allergy is to study monogenetic diseases which display a similar phenotype. Thus, we have focussed on a rare monogenetic autosomal recessive skin disorder - ichthyosis prematurity syndrome (IPS; also termed ichtyhosis congenita type IV). Key features of IPS are premature delivery (30-32 weeks), thick caseous desquamating epidermis and respiratory symptoms at the time of birth, which recover into a lifelong non-scaly ichthyosis with severe itching. Importantly, a striking finding in IPS is an extremely high number of peripheral blood eosinophils (in some cases >30%) and very high serum IgE levels (in some cases > 5000 kU/l).
IPS is almost exclusively present in a restricted area of middle Norway and Sweden, suggesting a recent founder mutation for this disease. Linkage of IPS has been mapped to a core region of 76 kb on chromosome 9q34. This region spans four known genes, but sequencing and expression analyses of these four genes have revealed no differences between patients and controls. Therefore, we performed microarray analyses comparing mRNA levels in skin biopsies and in cultured keratinocytes from patients and controls. Based on these experiments several candidate genes have been identified and detailed expression analysis of these genes and their corresponding gene products are currently under investigation. We strongly believe that identification of the IPS susceptibility gene and elucidation of its biological function may have ramifications for the understanding of the underlying mechanisms of atopy and allergy.

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**Pharmacogenetic Approaches in Asthma: Will personalized therapy guide asthma therapy?**

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Pharmacogenetic approaches in asthma may lead to reduced adverse events and improved therapeutic responses: key components of personalized medical approaches. There has been recent interest in pharmacogenetic responses to beta agonist and corticosteroid therapy in asthma. Initial data on $ADBR_2$ variation and reduced responses to short acting beta agonist therapy have led to studies evaluating $ADBR_2$ variation and responses to long acting beta agonists (LABA). These studies show inconsistent effects of ADBR2 variation on responses to LABA therapy although a recent analysis in a large retrospective and a large prospective cohort do not show an interaction with the Arg16Gly variation in $ADBR_2$ with LABA responses (Bleecker et al: ACCP, Chest, 2007 and Lancet, In Press, 2008). There have been fewer published studies evaluating corticosteroid responses.
Corticosteroids exert their anti-inflammatory action by binding and activating the cytoplasmic glucocorticoid receptor (GR) heterocomplex. We have re-sequenced 9 genes encoding components of the GR heterocomplex, and have genotyped 60 polymorphisms in these 9 genes in adult asthmatics randomized to once daily inhaled corticosteroid therapy. We observed significant associations for polymorphisms in the gene *STIP1* for baseline $FEV_1$, baseline $FEV_1$ as % predicted, and % change in $FEV_1$ after 4 and 8 weeks of corticosteroid therapy. We also observed significant associations between *STIP1* haplotypes and baseline $FEV_1$ and % change in $FEV_1$ after 4 weeks of therapy, while similar trends were observed for baseline $FEV_1$ e.g. % predicted and % change in $FEV_1$ after 8 weeks of therapy. An association between *STIP1* haplotype pairs and % change in $FEV_1$ after corticosteroid therapy was also observed. Our data suggest that genetic variations in the gene *STIP1* may be useful in predicting corticosteroid responses in asthmatics with reduced lung function. While these pharmacogenetic approaches require further development and replication, they offer the potential of more personalized medicine and individualized therapies in asthma. This pharmacogenetic approach is an example of the NIH roadmap goal for personalized medicine.

## 39
**Routes of Allergic Sensitization to Peanut Protein**

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**Background:** More than 90% of peanut allergic children react on their first known oral exposure to peanut. Recent data suggest cutaneous exposure as a route of sensitization. This study aimed to establish the relevant route of peanut exposure in the development of allergy.
**Methods:** A Food Frequency Questionnaire was administered to children with peanut allergy (PA) as well as to high-risk (egg-allergic) controls and non-allergic controls. Questionnaires were completed before subjects were aware of their PA status, avoiding recall bias. Questionnaires recorded maternal peanut consumption during pregnancy, breast-feeding and first year-of-life. Peanut consumption was determined amongst all household members, allowing quantification of environmental household exposure. The FFQ was validated for recall and also against a 7 day food diary.
**Findings:** Median weekly household peanut-protein consumption in the 133 PA cases was significantly elevated (18.8g) compared to 150 non-allergic controls (6.9g) and the 160 high-risk controls(1.9g). There were no differences in infant peanut consumption between groups. Differences in maternal peanut consumption during pregnancy (and lactation) were significant but become non-significant after adjusting for environmental peanut exposure. A dose-response relationship was observed between environmental (non-oral) peanut exposure and the development of PA, which was strongest for peanut-butter. Early oral exposure to peanut in infants with high environmental peanut exposure may have had a protective effect against the development of PA.

## 40
**Filaggrin Loss-of-Function Mutations, Pet and Dust Mite Exposure and the Risk of Eczema in Childhood**

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**Background:** Loss-of-function variants in the gene encoding filaggrin (*FLG*) are major determinants of eczema. We hypothesized that weakening of physical barrier in *FLG*-deficient individuals may modulate the effect of exposure to pets and dust mites.
**Methods:** Unselected birth cohort: participants were recruited prenatally and followed prospectively (3, 5 and 8 years). Eczema was defined as a physician-confirmed eczema on physical examination at any follow-up visit (UK diagnostic criteria). Pet exposure was ascertained based on cat or dog ownership at birth and frequency of contact with pets in early childhood. We collected dust samples at birth, age 3 and 5 years (mattress, bedroom floor and lounge floor) to determine mite, cat and dog allergen levels (ELISA), expressed as sum of exposures. Samples were genotyped for the variants 2282del4 and SNP R501X. Children with either (or both) variants were classified as having the mutant allele.
**Results:** 471 children of Caucasian origin had information on FLG genotype, pet ownership and contact and prospective assessment of eczema. Mutant alleles were present in 44 (9.3%). By age eight years, eczema was diagnosed in 132/471 children (28.02%). Amongst children with *FLG* mutation, the risk of eczema significantly increased amongst those with frequent cat contact, but decreased in those who owned a dog at birth. In the multivariate model which included *FLG* genotype, contact with cats in early childhood, dog ownership at birth, the interaction between *FLG* genotype with cat contact and dog ownership and child's gender, significant and independent associates of eczema were the presence of *FLG* mutation (OR 3.15, 95% CI 1.30-7.62, p=0.01), the interaction between *FLG* genotype and contact with cats (OR 5.67, 95% CI 1.00-32.25, p=0.05) and the interaction between *FLG* genotype and dog ownership (OR 0.05, 95% CI 0.004-0.64, p=0.02). Furthermore, logistic regression analyses indicated that amongst children with *FLG* mutation the risk of eczema significantly increased with the increasing Fel d 1 and Der p 1 exposure, but decreases with increasing Can f 1 levels. No such effect was apparent in children without *FLG* mutation.
**Conclusions:** *FLG*-deficient individuals should avoid cat in early life, but may benefit from having a dog.

## 41
**Endotoxin levels in house dust and early infant gut microbiota**

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*Statement:* An altered microbial exposure may underlie the increase of allergic disease in affluent societies. Early colonisation with Bifidobacterium (B) and Lactobacilli (L) have been postulated to prevent children from developing allergy, while *Clostridium (C) difficile* colonisation has been associated with allergic disease. Little is known of the association between environmental factors and gut flora characteristics, however.
*Methods:* Presence and amounts of species-specific and total bacterial DNA in infant faecal samples, collected at 1 week, 1 month and 2 months, were measured with real-time PCR in 37 Swedish children, followed prospectively to five years of age. Primers binding to DNA from *C difficile, B bifidum, B longum/infantis, B adolescentis, B breve, Bacteroides fragilis,* Lactobacilli group I (*L rhamnosus, L paracasei*, *L casei*) and Lactobacilli group II (*L. gasseri, L. johnsonii* group) were used. Carpet dust samples were collected during the child's first year of life and endotoxin levels were determined by a chromogenic Limulus assay.
*Results:* Significantly higher endotoxin levels were found in homes of children with more than three, as compared to one or less, detected Bifidobacterial species at 1 week and 1 month of age. Also, the relative amounts of *B longum/infantis* at 1 and 2 months of age correlated positively to the endotoxin levels (*rho*=0.39-0.42, $p<0.05$). In contrast, the endotoxin levels were inversely related to the relative amounts of *C difficile* at 1 month of age (*rho*=-0.49, p=0.01), with a similar trend at 2 months of age.

*Conclusions:* An enhanced microbial load in the home environment, determined as endotoxin levels in house dust, is associated with a more varied Bifidobacterial flora and less *C difficile* colonisation in early infancy, a gut microbiotal pattern previously associated with less allergy development.

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**Asthma Symptoms among Schoolchildren in Georgia: ISAAC Phases I, II and III**

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All existing contemporary data are indicating a relatively low prevalence of asthma in Georgia. In the ISAAC phase I investigation in Tbilisi, Georgia, it was reported that the prevalence of current wheezing was 3.7% and 5.9% in the 13/14 and 6/7 years-old children respectively. In phase II of the ISAAC performed in 2001-2002, there was an increase of the prevalence rate of asthma. It became 9.2% as defined by having symptoms of current wheezing during the last 12 months. In ISAAC phase II, 66% of individuals wheezing revealed bronchial hyperactivity BHR, 35.2% of the children had positive specific IgE, 37.0% had at least one positive prick skin test, and 57.4% of the children had a total serum IgE of >100 kU/l. In non-wheezers: 31.6% had a positive BHR, presence of a positive specific IgE occurred in 30.0%, at least one positive prick test was noted in 33.3%, and an elevated total serum IgE level of greater than 100 kU/l was noted in 51.7% of the children. ISAAC phase III showed that the prevalence rate of symptoms of current wheezing in 6-7 years-old and 13-14 years-old children in 2003 increased to 8.5% and 6.0% versus 5.9% and 3.7%, respectively compared to ISAAC phase I performed in Tbilisi, Georgia in 1995-1996. Our findings demonstrate that the epidemiological features of asthma in Georgia are changing, although the causes are still uncertain. The investigation of risk factors at local level might give additional information in order to undertake preventive measures.

**43**

**Sensitization to Ragweed Pollen in Switzerland: an emerging problem in the SAPALDIA (Swiss Study on air pollution and lung diseases in adults) cohort**

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**Background:** Ragweed *(Ambrosia)* pollen allergy has been observed to spread rapidly northwards from the south-western parts of Switzerland, in particular from Ticino and the Geneva area. Ragweed is known to be a very potent allergen with a long period of pollination. The object of this study was to identify the sensitization rate to ragweed pollen in the cross-sectional SAPALDIA cohort.
**Methods:** We evaluated 5828 participants of the SAPALDIA II survey (July 2001 to February 2003). The study centres were Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne and Wald. Specific IgE to common inhalant allergens (Phadiatop) and also to *Ambrosia artemisiifolia* were measured with a commercial assay (ImmunoCAP Pharmacia w1).
**Results:** 1761 subjects (30.14%) showed sensitization against common inhalant allergens. 378 subjects (7.8%) showed a positive IgE serum level to ragweed pollen indicating a sensitization. In those subjects with positive phadiotop even 365 subjects (20.75) were sensitized against ragweed. Only few subjects were monosensitized to ragweed pollen, while the majority was polysensitized also against mugwort.
**Conclusion:** Inhalant allergies are increasingly common in Switzerland with more than 30% of the population showing senisitization against common inhalant allergens. Our data also highlight a relevant level of ragweed pollen sensitivity not only in zones with already existing ambrosia presence, but also in other parts of Switzerland, especially among polysensitized patients. In these regions, ragweed pollen counts are still low at present but they might increase steadily in the future. Since ragweed serves as a relevant source of aero-allergenic pollen, the sensitization patterns and pollen load should be closely observed and measures for a possible eradication should be considered.

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**Endotoxin augments myeloid dendritic cell influx into the airways in allergic asthma patients**

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**Rationale**: Epidemiological studies have shown that exacerbation of asthma is modulated by environmental endotoxin. High levels of endotoxin are associated with asthma symptoms and the current use of asthma medication. However, the underlying mechanisms by which endotoxin modulates asthma is not completely understood.
**Objectives**: The aim of the study was to test whether endotoxin enhances the response of allergic asthmatics to allergen and to determine if this interaction is associated with increased numbers of antigen presenting cells in the airways.
**Methods**: Seventeen subjects with mild allergic asthma underwent segmental challenge with allergen, endotoxin, and the combination of both in three different lung segments via bronchoscopy. The cellular influx including monocytes, myeloid dendritic cells (mDCs), and plasmacytoid dendritic cells (pDCs) as well as the level of cytokines were assessed in BAL fluid obtained 24 hours after segmental challenge. Monocytes, mDCs, and pDCs were isolated and their capacity to induce T cell proliferation was determined.
**Measurements and Main Results:** Endotoxin enhanced the cellular response to allergen. The combination of allergen and endotoxin resulted in increased numbers of total cells, lymphocytes, neutrophils, eosinophils, monocytes, and mDCs as well as increased levels of LPS-binding protein, IL-1α, IL-6, and TNF-α in the BAL fluid compared to allergen alone. Isolated mDCs but not pDCs induced a strong T-cell proliferation in vitro.
**Conclusion:** Endotoxin augments allergic inflammation in the lung of asthmatics and induces an enhanced influx of monocytes and functionally active antigen-presenting mDCs into the respiratory tract.

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**Asthma deterioration induced by repeated low dose allergen exposure as a model to assess treatment effects on development of allergic airway inflammation**

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Repeated low-dose allergen inhalation challenge has been introduced as a method to mimic and standardize natural exposure to environmental allergens. On several consecutive weekdays, subjects with allergic asthma inhale fixed doses of allergen causing minimal bronchoconstriction. This is followed by increased airway hyperresponsiveness to direct bronchoconstrictors, and signs of airway inflammation such as elevations in exhaled nitric oxide ($F_ENO$) and biomarkers in sputum. Hence the model is particularly suitable to investigate early events occurring before the development of symptomatic asthma.

As the use of combination therapy in mild asthma is debated, the effects of formoterol alone and formoterol/budesonide combination inhaler on asthma deterioration induced by repeated low-dose allergen exposure was assessed (Clin Trial Reg No: NCT00288379).

Fifteen subjects with intermittent allergic asthma were exposed to repeated low doses of allergen on seven consecutive weekdays in a three-period, cross-over, double-blind, double dummy, comparison between formoterol Turbuhaler 4.5µg, budesonide 160µg /formoterol 4.5µg Turbuhaler, and placebo, each taken as two puffs 30 minutes after every allergen inhalation. Airway hyperresponsiveness to methacholine was assessed before and after each period. Spirometry and exhaled nitric oxide ($F_ENO$) values were obtained daily as well as diary card recordings of symptoms (0-10), short-acting $beta_2$-agonist use and evening $FEV_1$.

During placebo treatment, repeated low-dose allergen exposure resulted in significant increases in airway hyperresponsiveness (geom mean(CV) provocative dose of methacholine causing 20% fall in $FEV_1$($PD_{20}$): 397(98) µg before vs

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168(82) after, p=0.01), $F_ENO$ (mean(SD): 46(31) ppb before vs 73(46) after, p=0.006) and asthma symptom score (mean (SD): 0.39(0.55) before vs 0.68(0.67) after, p=0.003). Treatment with budesonide/formoterol abolished these changes (p=0.0098, p= 0.0002 and p=0.024 vs placebo, for $PD_{20}$, $F_ENO$ and symptoms, respectively) and significantly improved baseline $FEV_1$. Formoterol alone, while providing symptom relief, was no better than placebo in protecting against the allergen-induced increase in airway hyperresponsiveness and $F_ENO$.

It is concluded that signs of deteriorating asthma, provoked by low dose allergen, are prevented by as needed budesonide/formoterol but not by formoterol alone, highlighting the risk for masking of inflammation if long-acting beta agonists incorrectly are used as mono-therapy.

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### Increase in human neutrophil peptides 1-3 during rhinovirus-induced experimental asthma exacerbations

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Rhinoviruses have been identified as the major viral triggers of asthma exacerbations. Recently it has been shown that asthma is an independent risk factor for invasive pneumococcal disease. Human neutrophil peptides (HNPs) play an important role in antimicrobial host defence and are induced by inflammation. We hypothesised that HNPs are induced by rhinovirus infections and deficient in asthmatics compared to normals. Asthmatic (10) and healthy volunteers (15) were experimentally infected by nasal application of rhinovirus 16. Asthmatic patients developed significantly increased lower respiratory tract symptoms and airflow obstruction, whereas these features were not induced in healthy controls. Before, four days and six weeks after infection, bronchoalveolar lavage was obtained in all subjects and SLPI (secretory leucocyte protease inhibitor) and HNP 1-3 protein expression as well as expression of IL-8 and -15, ENA-78, GRO-α and TNF-α were determined in the supernatants. There was no induction of SLPI but there was a significant up-regulation of HNP 1-3 in asthmatics during infection (p=0.0026). In healthy controls there was no induction. Experimental rhinovirus infection lead to an up-regulation of HNP 1-3 in asthmatics only. HNP levels at day 4 were significantly higher in asthmatics than in controls (1.25 vs. 0.72 ng/ml, p=0.035). Considering the dilution effect of BAL excessive expression of HNP 1-3 may contribute to increased airway inflammation and epithelial damage. These data do not support the hypothesis that deficient HNP levels but possibly their excessive expression in asthma may be related to increased susceptibility to invasive pneumococcal disease.

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### Acute asthma in children presenting to an emergency room – role of infection versus allergy

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Although asthma is considered by many to be an allergic disease, acute attacks are most commonly precipitated by viral respiratory infection (VRI) particularly human rhinovirus (HRV). New evidence suggests the importance of VRI and HRV have been underestimated, as much higher infection rates have been reported using a new, improved approach to detect VRI and to detect and type HRV (Lee W-M et al PLoS ONE 2007;2:e966)( J Clin Microbiol. 2007;45;2626). The aim of the present study, therefore, was to compare rates of detection of VRI, including HRV, respiratory syncytial virus (RSV), parainfluenza virus (PIV), influenza virus (InfV) A and B and adenovirus (Ad), using previous methodologies with the new approach, and to evaluate immunological function both during and after the attack. We studied 90 children aged 2-16 yrs recruited on presentation to a children's hospital emergency room with asthma. The majority of the children had clinical asthma scores in the moderate to severe range and required supplemental $O_2$. A comprehensive evaluation was made of clinical history, haematological and immunological function, including plasma levels of IgG, IgG subclasses and specific and total IgE, both during the attack and after convalescence 6 weeks later. A nasal aspirate was obtained and analysed for the presence of respiratory viruses. The table compares data from the hospital's standard detection method with data from the improved methodology.

| | HRV | RSV | PIV | InfV | Ad | Other | Total with VRI |
|---|---|---|---|---|---|---|---|
| Old approach | 58 (64.4%) | 1 (1.1%) | 0 | 0 | 0 | Not tested | 58 (64.4%) |
| New method | 80 (88.9%) | 1 (1.1%) | 2 (2.2%) | 0 | 3 (3.3%) | 7 (7.7%) | 83 (92.2%) |

Note: (1) the new method was also able to detect other respiratory viruses, including coronavirus (CoV) 1 (1%), enterovirus (EnV) 3 (3.3%), human metapneumovirus (MPV) 3 (3.3%) and bocavirus 0; and (2) a dual infection occurred once with the old approach and 6 times with the new methodology: 3 HRV/Ad, 2 HRV/MPV and 1 HRV/RSV and a triple infection occurred once: HRV/EnV/CoV. We found no questionnaire, haematological or immunological evidence that allergy played a role in precipitating acute asthma in any child. In summary, conventional techniques missed many viral respiratory infections, particularly those with HRV. The very high percentages of VRI and HRV detected using the improved approach and the lack of evidence of a role for allergy suggest that the role of VRI in acute asthma in children has been underestimated.

## 48

### Nontypable *Haemophilus influenzae* (NTHi): A cause of bronchospasm and a target for therapy in subjects with smoking-related chronic airways disease (SRCAD)

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These studies examine the extent to which (i) those with both mild and severe SRCAD have detectable IgE anti-NTHi antibody, and (ii) an oral killed NTHi vaccine can reduce NTHi antigen within airways (monitored as serum IgG anti-OMP antibody) and bronchospasm. Three groups of smokers were studied: (1) recurrent acute wheezy bronchitis (20 disease [$FEV_1$ 2.6]/20 placebo [$FEV_1$ 2.0]), (2) moderate-severe COPD (17 disease [mean $FEV_1$ 0.5l] 9 controls), and (3) smokers without recognised COPD (32 active, 32 placebo).

(i) NTHi specific IgE antibody in SRCAD
Significant levels of IgE specific NTHi antibody was detected in the serum of subjects with SRCAD (Groups 1 and 2).

A. IgE antibody levels

| Recurrent wheezy acute bronchitis (Group 1) | | | | Chronic Obstructive Pulmonary Disease (Group 2) | | | |
|---|---|---|---|---|---|---|---|
| Patients (20) | | Controls (20) | | Patients (17) | | Controls (9) | |
| IgE ng/ml | IgE anti-OMP | IgE ng/ml | IgE anti-OMP (eu/ml) | IgE ng/ml | IgE anti-OMP (eu/ml) | IgE ng/ml | IgE anti-OMP (eu/ml) |
| 438 ± 100 * | 79 ± 14 ** | 401 ± 92 | 14 ± 4 | 992 ± 224 * | 257 ± 84 *** | 490 ±118 | 15±3 |

* <0.05; ** p<0.01; *** p<0.005 compared with corresponding placebo or control subjects.

(ii) Oral immunisation with NTHi vaccine

(a) Group 3. IgG anti-NTHi antibody over winter was used as a surrogate marker of NTHi accessing the terminal airways. Difference was found in the placebo subjects, with antibody levels increasing to 2-3 fold that observed in the immunised subjects ($p < 0.001$) and this increase was correlated with exposure to NTHi. Concomitant medications were less in the immunised subjects ($p < 0.05$) compared with the placebo subjects.
(b) Group 1. Immunised subjects had less ($p=0.02$) episodes of acute wheezy bronchitis.
**Conclusion:** Together (ie IgE antibody to NTHi in mild and severe SRCAD; effective reduction in intrabronchial NTHi antigen following oral vaccine; less episodes of wheezy bronchitis post vaccine) these results support the hypothesis that colonising NTHi contribute to bronchospasm, and that an oral NTHi vaccine has value in SRCAD management by reducing intrabronchial antigen concentration. The vaccine optimises normal aspiration of intrabronchial antigen for delivery to Peyer's patches.

## 49
### An Intranasal Steroid Reduces Eye Symptoms after Repetitive Nasal Challenges with Allergen

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**Background:** Clinical trials have shown that the use of intranasal steroids (INS) reduces the eye symptoms of patients with allergic rhinoconjunctivitis without signs of ocular steroid side effects. Thus, the mechanism, by which INS affect ocular allergic symptoms, requires further elucidation.
We previously demonstrated the existence of ocular symptoms in response to a nasal allergen challenge (NAC) suggesting the presence of a nasal-ocular reflex to eye symptoms in patients with allergic rhinitis. Our objective in this study was to test the hypotheses that priming of the nasal-ocular reflex occurs after repetitive NAC and that reduction of priming with treatment with an INS reduces eye symptoms.
**Methods:** We performed a double blind, placebo controlled, cross-over study with 17 subjects with seasonal allergic rhinitis who had previously demonstrated eye symptoms in response to NAC. Unilateral NAC was performed on 3 consecutive days after one week of treatment with either placebo or fluticasone furoate (FF). Nasal scrapings were obtained prior to each challenge to quantify eosinophils. The responses to NAC were monitored by diary of nasal symptoms, eye symptoms and by collection of bilateral nasal secretions.
**Results:** Eosinophils increased after NAC on placebo treatment. Repetitive NACs led to priming of the sneezing ($p=0.01$) and the nasonasal secretory reflex ($p=0.04$) and an increase in eye symptoms on placebo. Treatment with FF inhibited all the above allergen-induced responses including eosinophils ($p=0.03$), sneezes ($p<0.01$), contralateral secretion weights ($p<0.03$), and eye symptoms ($p<0.01$).
**Conclusions:** Our data confirm the presence of a nasal-ocular reflex following NAC and demonstrate reduction in this reflex by an intranasal steroid. These data support the contribution of a nasal-ocular reflex to the genesis of eye symptoms in patients with rhinoconjunctivitis and provides an explanation for the favorable effect of INS on eye symptoms. Since INS do not completely abolish eye symptoms of allergic rhinitis, direct contact of allergen with the conjunctiva combined with the nasal-ocular reflex is responsible for the eye symptoms of allergic rhinoconjunctivitis.

## 50
### Changes in central nervous processing of histamine-induced itch in atopic eczema

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Itch is a multidimensional sensation and the major symptom of allergy. We identified dynamic brain activation patterns of histamine-induced itch using alternating temperature modulation in healthy volunteers. The following study evaluated this biphasic stimulus model in lesional (LS) and non-lesional (NLS) atopic eczema (AE) skin.

In 10 right-handed male patients with AE (age 28±4.3y) 1% histamine dihydrochloride was applied on the right forearm in randomized order on LS or NLS, respectively. Subsequent thermal modulation (Medoc TSA II) was performed in rapid alternating order from 32°C (warm) to 25°C (cold); each temperature block lasted 20s. Subjective intensity and qualitative itch ratings were recorded using a computerized visual analog scale (VAS) and the Eppendorf Itch Questionnaire (EIQ). Healthy skin (HS) of 9 age-matched volunteers served as control.

All subjects reported localized itch sensations without pain; mean VAS itch intensity was significantly ($p<0.0001$) higher during the relative cold (55.2±8.3% (LS); 48.6±8.2% (NLS)) compared to the relative warm blocks (36.0±7.3% (LS); 33.7±7.6% (NLS)). Also, mean EIQ-ratings of the cold blocks were significantly higher. LS and NLS showed a delay in itch response compared to HS. Itch intensity was perceived highest in LS, followed by HS and NLS; emotional EIQ-ratings were rated highest for LS, followed by NLS and HS.

Conclusions: Short term moderate temperature decrease induced reproducible, significant enhancement of histamine-induced itch with the strongest effect in LS. The differences in itch intensity and itch kinetics between healthy volunteers and non-lesional skin in patients and the reduced initial itch intensity in patients point towards ongoing central inhibitory activity in AE. Consecutively itch is amplified in the patient group and shows a longer duration and higher emotional load. This stimulus paradigm is now used for fMRI investigation of the cerebral processing of itch in AE patients.

## 51
### Eczema Herpeticatum Recidivans

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Eczema herpeticum (EH) is defined as disseminated Herpes simpelx Virus (HSV) infection of an eczematous skin disease, which in clinical reality is almost exclusively atopic dermatitis (AD). High serum IgE and early onset of the underlying AD are established risk factors. Many patients have one episode of EH only (EHP), whereas some patients suffer from securrent EH (EHR). This multicenter study was performed to identify clinical risk factors for EHR.
A total of 217 patients with a serum IgE of 3450±6821 kU/l and a SCORAD of 44±18, diagnosed from 1996 to 2006 with EH, were included in the analysis. Intrinsic AD was diagnosed in one patient only.
Herpes blisters were present within the eczematous skin lesions only, indicating a need for clinically active AD lesions to develop EH. The frequency of EHR (26%) was markedly higher than reported in previous decades, which demonstrates a change of the clinical disease spectrum. The EHR patients showed a higher serum IgE, more eosinophils and an earlier onset of the underlying AD as compared to EHP. In addition, they used steroid ointment less frequently.
In conclusion, EHR patients exhibit the established risk factors for EH (severe, uncontrolled AD) in a more pronounced manner and may be described as being even 'more atopic' than EHP patients.

# Abstracts

## 52
### Establishment of food provocation network in Japan

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The gold standard of diagnosis of food allergy is made by the double blind placebo-controlled food challenge (DBPCFC), but it requires a lot of work to prepare the materials for DBPCFC. From the year of 2001, we have established and maintained a network of food provocation using a same protocol and a blinded food provocation kit developed in the previous study for the purpose of dissemination of food provocation in Japan. The blinded food provocation kit consisted of 4-6g dried powder of each food (whole egg, cow's milk, wheat, and soy bean) and 120g strawberry puree. Dried powder of each food was prepared by spray dry or freeze dry method. Strawberry puree was the best material for masking smell and color of provocation food to perform blind challenges. One thousand five hundred ninety provocations were performed in the total 30 hospitals joined to the study (mean age: 5.0 ± 0.2 y). Of 1590 provocations, whole egg provocation tests were 644 cases, egg yolk 152 cases, cow's milk 442 cases, wheat 238 cases, soybean 114 cases, respectively. The food challenge procedure was carried out in admission along the AAAAI DBPCFC manual (JACI 82:986, 1988). Starting initial dose from 5% of total volume, provocation doses were doubled every 15 min. The evaluation was made by clinical scoring system. Positive reactions were observed in 753 cases out of 1590 cases (whole egg: 394/644 (61.0%), egg yolk: 37/152 (24.3%), cow's milk: 212/442 (48.0%), wheat: 91/238 (38.2%), soy bean: 19/114 (16.7%)). The most common symptom was seen in the skin (59.9%), followed by GI symptom (33.2%), and respiratory symptom (28.6%). Whole egg and soybean tended to induce more GI symptom than cow's milk and wheat (44.9%and 31.6% vs 20.3% and 16.5%), whereas cow's milk and wheat tended to induce more respiratory symptom than whole egg and soybean (37.3% and 38.5% vs 22.3% and 15.8%)). The establishment of food provocation network could be achieved by the grant from the Ministry of Health and Welfare. This trial enhanced the dissemination of food provocation test in Japan in the past six years.

## 53
### When anti-acid treatment goes sour: Food allergy induction in the whole system

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**Background:** Anti-acid drugs induce sensitization against food antigens. We have confirmed this Th2-response on humoral and cellular levels. In the case of the anti-acid drug sucralfate, food allergy might be induced by blocked protein digestion due to pH-elevation and, additionally, by comprised aluminium compounds, which may contribute to an allergic response.
**Objective:** In murine studies we examined the outcome of treatment with sucralfate when applied parenterally to investigate the effect of its aluminium compound. Secondly, we checked the influence of sucralfate when given orally on immunological and morphological parameters in the intestine.
**Methods**: BALB/c mice were treated subcutaneously with codfish extract plus sucralfate. In a second experiment, mice got this mixture via the oral route. Antibodies were examined in ELISA, RBL-assay and Western blot. In the feeding experiment, quantitative morphological analysis of the intestine was performed by design-based stereology, with focus on epithelium, lamina propria, smooth muscle cells, eosinophils and $CD3^+$ cells. Histological samples were analyzed after H&E-, PAS- and Congo red-staining. Immunohistochemistry was performed for detection of $CD3^+$ cells.
**Results:** Sucralfate was perfectly capable of inducing a Th2-response on the subcutaneous route, confirmed by allergen-specific IgE and its biological activity in RBL- and skin tests. Verifying the role of alum as foreign substance, histological examinations of the subcutaneous injection sites revealed huge granulomas. All these proofs of a humoral Th2-response could be reproduced via the oral route. In addition, histological examination of the intestinal sections after sucralfate-gavages showed reduced thickness of villi in duodenum. Proving the involvement of immune cells, increased goblet cell mucus was seen in the caecum, and higher eosinophil/$CD3^+$ ratios were found in stereological analysis of the intestine. Additionally, morphological changes like decreased thickness of the epithelium and thinner smooth muscle cell layer underlined the food allergic pattern in sucralfate-treated mice.
**Conclusion:** Anti-acid drugs induce a higher risk for sensitization against food antigens. In this study we confirm that for sucralfate this is due to pH-elevation and adjuvant activity. Importantly, this medication also changes the morphology and immunology of the intestine in a way typical for food allergy.
**Support**: Hertha-Firnberg stipend T283-B13, project P18238, grant SFB-F1808-8 of the FWF; grant SFB/TR 22 of the DFG.

## 54
### Sensitization to Hymenoptera venom in patients with anaphylaxis to Hymenoptera stings and mastocytosis

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**Background:** There are reports on patients with mastocytosis and systemic anaphylactic reaction (SAR) to a Hymenoptera sting (HS) in whom sensitization to Hymenoptera venom (HV) could not be demonstrated. It was assumed that SAR in these patients resulted from toxic action of HV on an increased number of mast cells ("pseudo-allergy").
**Patients and Methods:** We retrospectively included 79 patients with systemic and/or cutaneous mastocytosis (diagnosed by standard criteria) and a history of SAR to a HS. 969 consecutive patients with SAR after a HS with baseline serum tryptase concentration <11.4 µg/l (95th percentile in normals) and no indication of mastocytosis served as controls. HV sensitization was assessed by titrated skin tests with honey bee venom (HBV) and yellow jacket venom (YJV) (skin prick test up to 100 µg/ml, if negative intradermal test with 1 µg/ml) and/or by measuring specific serum IgE to HBV and YJV by CAP-FEIA (positive: > 0.35 kU/l).
**Results:** 79.8% of mastocytosis patients, but only 16.3% of the control patients ($p < 0.001$) had a history of a severe SAR to HS (full shock, requirement of resuscitation). HV-specific IgE antibodies were detected significantly less frequent in mastocytosis patients than in controls: to HBV in 42.3% versus 56.7% ($p=0.017$), to YJV in 63.6% versus 78.4% ($p=0.005$). No significant differences between patients and controls were found with respect to skin test reactivity to HV. When results obtained with any venom at skin tests or IgE measurements were pooled, sensitization was demonstrable in 94.8% of mastocytosis patients and 98.6% of control patients (not significant); double sensitization to both HBV and YJV was less frequent in patients with mastocytosis (63.0%) than in control patients (68.0%; not significant).
**Conclusion:** HV-specific IgE-antibodies were significantly less often detectable in patients with mastocytosis than in controls, but there was no such difference with regard to skin test results. Overall frequency of sensitization to HV did not differ significantly between patients with mastocytosis and controls. "Pseudo-allergy" seems not to be a cause of sting anaphylaxis in patients with mastocytosis.

## 55
### Stanozolol Therapy for Hereditary Angioedema

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The goals of treatment for hereditary angioedema (HAE) include reversal of acute attacks and the long-term prevention of spontaneous episodes. Several drugs are currently available for such therapy. These include antifibrinolytic agents (ε-

aminocarpoic acid, tranexamic acid), fresh frozen plasma, and anabolic steroids (stanozolol, danocrine). Five new drugs address the biochemical basis of this disorder. Ecallantide (Dyax) inhibits kalikrein and bradykinin production. Icatibant (Jerini) is a bradykinin 2 receptor antagonist. Cinryze (Lev pharmaceuticals) and Berinert P (CSL Bhering) are pasteurized, ultrafiltered human C 1 inhibitor (C1 INH), and Rhucin (Pharming) is recombinant human C1 INH.

The administration of methyltestosterone and attenuated androgens (i.e. oxymethalone, danazol, and stanozolol) reduces the frequency and severity of spontaneous attacks of HAE. Although the efficacy of the anabolic steroids is well established, the mechanism for this effect remains unclear. Stanozolol is metabolized in the liver and with its administration there in an enhancement of C1 INH protein production. However, the therapeutic dose, usually 0.5-2mg daily, reduces the HAE episodes without increasing the serum concentration of C1 INH into the normal range. Higher dosages, administered more frequently, are associated with toxicity.

Since 1966, 37 HAE patients at the Brigham and Women's Hospital have been administered steroid with impeded androgenic effect (17-α-alkylated anabolic agents). Four were administered methyltestosterone and subsequently were treated, along with 33 other patients, with oxymethalone. All have been subsequently administered stanozolol for a period up to 40 years for long-term chronic prophylaxis. Most patients achieve symptomatic control and decrease the dose of stanozolol according to our published tapering protocol. At the minimum effective dosage, the mean maximum values for hepatic transaminases function were within the normal range. Minimal adverse drug effects occurred during short-term and long-term therapy. When biochemical abnormalities occurred, they were reversible in most instances with reduction in stanozolol dosage.

Until the newer agents are approved for treatment, anabolic steroids with impeded androgenic effects are the most readily available drugs for chronic use. Such agents are most effectively adjusted by periodic patient assessment, including monitoring of focused laboratory tests.

## 56

### Maternal undernutrition during pregnancy results in persistent alterations in lung gene and miRNA expression: Evidence for fetal programming of lung development

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**Background:** Disordered growth during early life has been shown to result in chronic diseases such as diabetes and coronary disease in later life. Environmental factors such as unbalanced nutrition before birth result in metabolic and structural adaptations that lead to persistent modifications to offspring phenotype, i.e. fetal programming. There is evidence that respiratory disease is also influenced by fetal programming. Asthma has been linked epidemiologically with markers of fetal growth such as anthropometric measurements at birth. Reduced fetal growth and duration of gestation are associated with impaired lung development in children. Airway function at birth is also a significant predictor of asthma, adult lung function, and possibly COPD. We have investigated the effects of maternal under-nutrition on lung development in a rat model of maternal protein restriction.
**Methods:** On confirmation of pregnancy female Wistar rats were allocated to either control (C, 18% casein) or protein restricted (PR, 9% casein) diet. Lung tissue was harvested at 120 days of age from the offspring (C, n=7; PR, n=9). Total RNA was extracted from ~100mg of lung. Target and reference (rActb, rB2m and rUbc) gene expression assessed using quantitative RT-PCR. Alterations in lung micro-RNA expression were assessed using miRXplore™ microarrays.
**Results:** Expression of 11β-hydroxysteroid dehydrogenase D1 and 2 (regulators of glucocorticoid action), Insulin like growth factors 1 and 2 (regulators of fetal growth) and p53 (regulator of lung apoptotic/cell proliferation balance during alveolar development) were significantly increased in response to PR (1.5-6.4 fold, $p<0.05$, Mann-Whitney U). 15 miRNA species were up-regulated (1.8-16.1 fold increase in expression) and 13 miRNAs down-regulated (1.8-13.7 fold) in response to maternal PR.
**Conclusions:** The identification of persistent alterations in gene and miRNA expression in response to maternal protein restriction in rat lungs at 120 days of age shows that *in utero* environmental exposures result in altered lung development and persistent phenotypic changes. Significant alterations in expression of miRNA may indicate that epigenetic programming of miRNA expression is a potential novel mechanism of fetal programming. Further work will seek to establish the role of epigenetics (DNA methylation) in persistent alterations of gene and miRNA expression in the lung.

## 57

### Opposite Effects Of Day-Care Attendance On Atopic Sensitization And Wheezing Among Children With Different Polymorphism In TLR2

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**Background:** Variation in TLR2 gene *(TLR2/−16934)* is associated with the frequency of allergic diseases amongst farmers' children (T allele carriers are less likely to have allergies compared to children with genotype AA); no such association is found amongst children not living on farms (JACI 2004;113:482-8). We hypothesized that the same genetic variant which confers protection in the farming environment may be associated with a reduced risk of allergic phenotypes amongst urban children exposed to microbial agents in the day-care.
**Methods:** Population-based birth cohort; participants were recruited prenatally and reviewed at ages 1, 3, 5 and 8 years (±4 weeks). Information on parentally-reported symptoms and day-care attendance was collected using validated questionnaires. Sensitization was ascertained by skin prick testing. Genotyping was performed using the Single Base Extension method.
**Results:** 717 children of mixed European ancestry provided samples for genotyping; 496 attended day-care. Genotype frequencies were in Hardy-Weinberg equilibrium and consistent with other populations (AA-22.3%, AT-50.5%, TT-27.2%). There was no association between *TLR2/−16934* and sensitization, current wheeze or atopic wheeze in the whole population. However, day-care significantly modified the association between allergic phenotypes and *TLR2/−16934*. This analysis suggested an increased risk of developing sensitization amongst children with AA genotype who attended day-care; in marked contrast, T allele carriers who attended day-care were less likely to be sensitized at each time point. The longitudinal analysis demonstrated significant association of atopy with the interaction between the *TLR2/−16934* polymorphism and day-care attendance (OR 0.43, 95% CI 0.20-0.95, p=0.04). Furthermore, in the multiple logistic regression day-care-*TLR2/−16934* interaction was significantly associated with atopic wheezing at age 8 years (OR 0.19, 95%CI 0.04-1.00, p=0.05).
**Conclusions:** Our data provide evidence for the opposite effect of day-care on sensitization and wheezing among children with different variants of the *TLR2* gene. Children with T allele for *TLR2/−1693* may benefit from attending day-care, whereas for AA homozygotes being cared for at home may prove beneficial. These results suggest that in asthma and allergic diseases, genetic predisposition has to be taken into account when assessing the effect of environment, and vice-versa, relevant environmental exposures need to be factored into the genetic association studies.

## 58

### The Gene Encoding *SMYD3*, a Histone Methyltransferase, is Associated with Asthma in Ethnically Different Populations

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# Abstracts

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Very recently, the genome-wide association approach has proved to be both feasible and useful in terms of elucidating solid candidate genes for complex diseases, including asthma. As part of our 'Genome-Wide Association Studies of Asthma in Populations of African Descent', we have genotyped 655,352 single nucleotide polymorphisms (SNPs) using the Illumina HumanHap650Y BeadChip on 498 African American asthma cases and 500 African American non-asthmatic controls. The genome-wide panel included 195 tagging SNPs in the *SMYD3* gene localized on chromosome 1q44. Chromosome 1q44 is a locus for which linkage for a variety of autoimmune diseases and diseases of inflammation (*e.g.*, rheumatoid arthritis, lupus diabetes, irritable bowel syndrome, psoriasis), and also asthma, has been reported. A single SNP analysis was performed with the Armitage trend test using PLINK software to estimate the overall significance of association between alleles and asthma. Five *SMYD3* SNPs showed significant associations with asthma ($P$=0.002-0.01). To replicate these findings, we genotyped the same five markers in three independent and ethnically distinct populations. One marker (rs2791391) was significantly associated with asthma in a group of 184 asthmatic cases and 184 healthy controls from Colombia ($P$=0.036). Two other markers (rs10802267, rs7530952) were significantly associated with asthma in 677 European American asthmatics and their family members ($P$=0.011 and $P$=0.029, respectively). We did not observe similar associations for the *SYMD3* markers and asthma in 762 asthmatics and their family members from Bahia, Brazil. The *SMYD3* gene encodes a novel histone methyltransferase that plays a role in transcriptional regulation. *SMYD3* encodes a 428 aa protein that directly trans-activates the telomerase reverse transcriptase (hTERT) gene, which is induced by natural antigen in allergen specific T lymphocytes in bronchial asthma. Collectively, our findings suggest that polymorphisms in *SMYD3* are associated with asthma in ethnically diverse populations, and may represent an important candidate gene for this complex trait.

## 59
### Genetic Pathway Analysis in Severe Asthma

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Most studies on the genetic basis of asthma have focused on identifying genes that confer asthma susceptibility and regulate pharmacogenetic responses. Recently, there has been increased interest in understanding the physiologic and genetic factors that interact to determine disease expression and asthma severity. SARP is an NIH funded consortium that has characterized more than 800 asthmatics including more than 300 with severe asthma. We are currently performing a genome wide association study on this cohort that is supported by the NHLBI STAMPEED program. We have also evaluated several inflammatory pathways and asthma susceptibility genes to determine their role in determining asthma severity. ADAM33 has been extensively resequenced and genotyped with results showing statistical relationships with pulmonary function ($FEV_1$) and health care utilization. Eight genes from the IL4 pathway (*IL4, IL13, IL4R, IL13RA1, IL13RA2, GATA3, STAT6, JAK1*) with 138 SNPs have been genotyped to determine their role in asthma severity. Associations with severity, lung function, and health care utilization have been found. Finally, we have genotyped 4 genes (TNFα, TNFRSF1A, TNFRSF1B, ADAM17) with 89 SNPs from the TNF pathway in the SARP population. Despite previous reports, there was no association with SNPs in TNFα; however, multiple SNPs in both TNF receptor genes showed significant evidence for association with bronchodilator reversibility and bronchial hyperresponsiveness to methacholine. (p values ranged from 0.001 to 0.04). In conclusion, prior to completion of the GWAS study, we have evaluated, using haplotype tagging SNPs, genes in several inflammatory pathways and have identified gene variants that are related to severe asthma phenotypes. This approach should lead to improved understanding of biologic and genetic mechanisms that influence asthma severity.

## 60
### Early aberrant antibody responses of aeroallergen-sensitised people to subclinical bacterial infection

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**Background**: Studies with polyclonal stimulation have shown that the immune systems of infants who develop inhalant allergies are slow to mature. There is however no information on non-allergenic responses to antigens presented in the respiratory tract other than those induced by acute infection.
**Aim**: To examine associations of allergy with differences in immune responses to a ubiquitous non-allergenic antigen presented repeatedly to the respiratory mucosa without clinical infection.
**Method:** Quantitative titres of IgG subclass and IgE antibodies to the purified conserved P6 protein antigen of the common colonising nontypable Haemophilus influenzae bacteria were measured in children and adults with and without allergies to prevailing aeroallergens.
**Results:** About 30% of school-aged children and adults with aeroallergy, predominantly to the house dust mite, had anti-P6 antibodies of the Th2-associated IgG4 subclass while these were not found in non-allergic people. There was strong sex bias with IgG4 being more prevalent in males. IgG1 antibodies were abundant and did not show the same degree of difference associated with allergy and sex. When the development of the response was examined it showed that infants who were destined to become allergic had a strikingly slow development of IgG1 antibodies shown at 2 years and developed IgG4 responses detectable at 5 but not 2 years. IgE antibodies could be detected in 40% of allergic subjects reaching titres of over 10 ng/ml, levels in the range of responses to major allergens. Children recovering from exacerbations of asthma showed increased anti-P6 IgE compared to their titres on admission.
**Conclusions**: The response of people with aeroallergies, especially males, to a bacterial antigen typically presented in repeated subclinical infections has a Th2 component. Its marked nature and early appearance in infancy suggests that it shares a common aetiology with responses to allergens rather than being a consequence. It is also possible that responses to repeated subclinical bacterial colonisation could have a role in the development, maintenance or manifestation of allergic sensitisation.

## 61
### Structure and mechanism of human leukotriene $C_4$ synthase, an integral membrane protein involved in bronchial asthma

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Leukotriene $C_4$ synthase (LTC4S) is an integral membrane protein that catalyzes conjugation of GSH with leukotriene $A_4$ (LTA4) to form $LTC_4$, i.e. the committed step in the biosynthesis of cysteinyl-leukotrienes, powerful lipid mediators of inflammation, immune modulation, and immediate hypersensitivity reactions. In particular, cysteinyl-leukotrienes are powerful smooth muscle contracting agents in the respiratory tract and microcirculation, which in turn leads to bronchoconstriction and edema formation. Insights to the structural basis and molecular mechanisms of $LTC_4$ synthesis is necessary for understanding cysteinyl-leukotriene biosynthesis and regulation.

Here we present the crystal structure of human LTC4S at 2.15Å resolution. The structure reveals a homotrimer, where each monomer is composed of four transmembrane segments. The structure of the enzyme in complex with substrate reveals that the active site keeps GSH in a horseshoe-shaped conformation, and effectively positions the thiol group for activation by a nearby Arg at the membrane–enzyme interface. In addition, the structure provides a model for how the $\omega$-end of the lipid co-substrate $LTA_4$ is held in place at one end of a hydrophobic cleft, guiding proper alignment of the reactive epoxide at the thiol of GSH. Our results also suggest that the observed binding and activation of GSH might be common for a family of homologous proteins important for inflammatory and detoxification responses.

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The crystal structure of LTC4S and accompanying insights to its molecular architecture and mechanism, will offer novel opportunities for structure-based design of drugs for treatment of several acute and chronic inflammatory diseases of the cardiovascular, renal and respiratory systems, in particular asthma.

**Acknowledgements:** This study was financially supported by the EU FP6 projects EICOSANOX (LSHM-CT-2004-005033) and the E-MeP (European Membrane Protein Consortium), the Swedish Research Council and the Swedish Cancer Society.

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### Leukotriene $E_4$ activates peroxisome proliferator activated receptor gamma and potently induces prostaglandin $D_2$ generation by human mast cells

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Cysteinyl leukotrienes (cys-LTs) are potent inflammatory lipid mediators. Cys-LTs amplify mast cell (MC) proliferation at inflamed mucosal surfaces. Leukotriene (LT)$E_4$ is the most stable and abundant cys-LT in vivo. Although only a weak agonist of established G protein coupled receptors [GPCRs] for cys-LTs, $LTE_4$ is unique for its ability to induce bronchial eosinophilia and to potentiate airway hyperresponsiveness (AHR) by a cyclooxygenase (COX)-dependent mechanism in vivo. We now report that $LTE_4$ induces signaling and activation responses from human mast cells (hMCs) involving cooperation between MK571-sensitive G protein-coupled receptors (GPCRs) and peroxisome proliferator activating receptor (PPAR)$\gamma$, a nuclear receptor for dietary lipids and endogenous lipid mediators. $LTE_4$ exceeds the potency of $LTD_4$ for inducing proliferation and chemokine generation by LAD2 cells, a human MC sarcoma line. $LTE_4$ strikingly upregulates COX-2 expression and induces prostaglandin $D_2$ ($PGD_2$) generation by LAD2 cells, whereas $LTD_4$ fails to induce these responses despite exceeding the potency of $LTE_4$ for calcium flux. $LTE_4$ causes phosphorylation of extracellular signal regulated kinase (ERK), pRSK90, and CREB. ERK activation in response to $LTE_4$, but not to $LTD_4$, is resistant to inhibitors of phosphoinositol 3-kinase. $LTE_4$-mediated COX-2 induction, $PGD_2$ generation, and ERK phosphorylation are all sensitive to interference by the PPAR$\gamma$ antagonist GW9662, and to targeted knock-down of PPAR$\gamma$; each of these responses were also sensitive to the $CysLT_1$ antagonist MK571. Physiologic responses of human airways to $LTE_4$ in vivo may reflect the unique ability of this eicosanoid to induce transcription of chemokines and COX-2 in MCs through complementary GPCR- and PPAR$\gamma$-dependent pathways.

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## 63

### Identification of a critical link between TRAIL and CCL20 for the activation of $T_H2$ cells and the expression of allergic airway disease

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The role of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in $CD4^+$ T helper 2 lymphocyte ($T_H2$) mediated immune responses is unknown. Here we characterize the development of hallmark features of allergic airway disease in wild-type and $TRAIL^{-/-}$ mice, or after pulmonary exposure to recombinant (r)TRAIL and short interfering RNA. TRAIL was abundantly expressed in the respiratory epithelium of allergic mice and inhibition of TRAIL resulted in impaired production of CCL20 and subsequent homing of myeloid dendritic cells and $CCR6^+CD4^+$ T cells to the airways. Attenuated homing limited $T_H2$ cytokine release, inflammation, airways hyperreactivity (AHR) and expression of signal-transducer-and-activator-of-transcription 6 (STAT6) whereas activation of STAT6 by IL-13 restored AHR in $TRAIL^{-/-}$ mice. Administration of rTRAIL was sufficient to induce characteristic features of asthma in an IL-13 dependent manner. Furthermore, asthmatics had elevated TRAIL levels in their sputum and rTRAIL induced production of CCL20 in primary bronchial epithelium cells. The function and expression of TRAIL in the airway epithelium identifies this molecule as a novel and accessible target for the treatment of asthma.

## 64

### Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease

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**Background:** Excessive production of airway mucus is a cardinal feature of bronchial asthma and chronic obstructive pulmonary disease (COPD) and contributes to morbidity and mortality in these diseases. IL-13, a Th2-type cytokine, is a central mediator in the pathogenesis of bronchial asthma, including mucus overproduction However, the precise mechanism of mucus hyperproduction induced by IL-13 has been unresolved.
**Methods:** To identify genes associated with mucus production, we subjected freshly prepared primary human tracheal epithelial cells cultured with IL-13 to microarray analysis. We generated asthma and COPD model mice by inhalation of ovalbumin or intratracheal administration of pancreas elastase, respectively. Expression of pendrin into NCI-H292 cells (human lung carcinoma cell line) was performed by transfection of the plasmid coding EGFP and pendrin. For expression of pendrin *in vivo*, Sendai virus vector-coding pendrin was administered transnasally into mice.
**Results:** Among the IL-13–inducible genes identified by the microarray analysis, we focused on the *PDS* (*SLC26A4*) gene encoding pendrin, an anion transporter, identified as the defective product in Pendred's syndrome characterized by deafness and goiter. Pendrin was highly induced by IL-13 in airway epithelial cells. In both asthma and COPD mouse models, pendrin was up-regulated at the apical side of airway epithelial cells in association with mucus overproduction. NCI-H292 cells stably expressing pendrin induced expression of MUC5AC, a major product of mucus in asthma and COPD, and knockdown of pendrin by siRNA attenuated MUC5AC production. Finally, the enforced expression of pendrin in airway epithelial cells *in vivo*, utilizing a Sendai virus vector, rapidly induced mucus overproduction in the lumens of the lungs together with neutrophilic infiltration and enhanced airway hyperreactivity in mice.
**Conclusions:** Pendrin is a critical mediator of mucus production in airway epithelial cells and is a therapeutic target candidate for bronchial asthma and COPD.

## 65

### The mast cell-restricted, tetramer-forming tryptases mMCP-6 and mMCP-7 are critical mediators in a mouse model of inflammatory arthritis

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**Background:** Increased numbers of $\beta$ $tryptase^+$/$heparin^+$ mast cells (MCs) have been detected in the synovium of patients with rheumatoid arthritis (RA). Mouse MC protease (mMCP) 6 and mMCP-7 are the corresponding tryptases stored in the secretory granules of mouse MCs ionically bound to heparin-containing proteoglycans. Although we and many others obtained data implicating a prominent involvement of MCs in RA and relevant animal models, no direct evidence for a MC-restricted tryptase in the pathogenesis of inflammatory arthritis has been shown. Our creation of transgenic mice that lack heparin and different combinations of mMCP-6 and mMCP-7 allowed us the opportunity to evaluate the roles of MC-restricted tryptase/heparin complexes in an animal arthritis model in a manner that previously was not possible.
**Methods:** The methylated bovine serum albumin/interleukin-1$\beta$ (mBSA/IL-1$\beta$) experimental protocol described by Lawlor and coworkers was used to induce arthritis in four mouse strains. Mice were injected intra-articularly into each knee joint with 10 µl of a 20 mg/ml solution of mBSA. Recombinant IL-1$\beta$ (250 ng) was then injected subcutaneously into the rear footpad. Mice were sacrificed 7 days after the initial injections, and standard histochemistry methods were used to assess joint pathology.

# Abstracts

**Results:** Histological analysis of the knee joints from mice affected by mBSA/IL-1β-induced arthritis revealed numerous activated MCs that had exocytosed the contents of their secretory granules. Arthritis was markedly reduced in heparin-deficient mice, implicating a prominent role for an undefined protease/heparin complex. The knee joints of mBSA/IL-1β-treated mMCP-6$^{+}$/mMCP-7$^{-}$ and mMCP-6$^{-}$/mMCP-7$^{+}$ C57BL/6 mice showed arthritis of comparable severity. In contrast, arthritis was markedly reduced in mMCP-6$^{-}$/mMCP-7$^{-}$ C57BL/6 mice.
**Conclusion:** The accumulated data implicate significant pro-inflammatory roles for MC tryptase/heparin complexes in the mBSA/IL-1β mouse model of RA. Because mMCP-6 and mMCP-7 can compensate for each other in this experimental disease model, it is now apparent that one must knock out both mouse tryptase genes to uncover the prominent roles of these proteases in joint inflammation and destruction. Based on our findings, selective inhibition of β tryptases may have therapeutic potential in the treatment of RA.

### 66
### Interfering with Histamine-Mediated Signaling Results in Significant Protection Against Severe Malaria in Mice

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From the inoculation of *Plasmodium* parasites through *Anopheles* mosquito bites until the blood-stage development of the parasite, a hallmark of the host response is an inflammatory reaction characterized by elevated histaminemia levels and histamine concentrations in tissues. Since histamine is endowed with pro-inflammatory and immunosuppressive activities, we postulated that this vaso-active amine participes to malaria pathogenesis. Combining genetic and pharmacologic approaches, we found that histamine binding to H1- and H2-R increases the susceptibility of mice to *Plasmodium* infection. To further assess the role of histamine in malaria pathogenesis, we used histidine decarboxylase deficient (HDC$^{-/-}$) mice which are free of histamine. HDC$^{-/-}$ mice were found highly resistant to severe malaria whether mice were infected through mosquito bites or with infected erythrocytes. HDC$^{-/-}$ mice were resistant to *Plasmodium* parasites from two different lethal strains, *Plasmodium berghei* ANKA (*PbA*) which triggers cerebral malaria (CM) and *Plasmodium berghei* NK65 (*Pb NK65*) which causes death without neurological symptoms. In CM, resistance of HDC$^{-/-}$ mice was characterized by a preserved blood brain barrier integrity, absence of aggregates of infected erythrocytes in the brain vessels, and a lack of sequestration of CD4 and CD8 T cells.
We identified here a novel inflammatory pathway resulting in histamine production that discriminates between malaria-susceptible and resistant mice. Understanding the biological effects of histamine during infection may lead to novel therapeutic strategies to alleviate the severity of malaria.

### 67
### Age related changes in regulatory T cell markers and toll-like receptor (TLR)-mediated innate mediated responses in allergic and nonallergic children

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**Background:** Disordered development of regulatory pathways and innate immune activation are implicated in the pathogenesis of allergic diseases. This study examined for early differences these pathways in allergic and nonallergic children, and how this changes over the first 6 months of life.
**Methods:** Markers of regulatory immune function (CD4+CD25+ T cells and FOXP3 expression) and innate toll-like receptor function (cytokine responses to TLR4 and TLR2 ligands) were compared at birth and at 6 months of age in children with subsequently defined allergic outcomes (diagnosed allergic disease and sensitisation at 1 or 2.5 years of age). Polyclonal T cell responses to (phytohaemaglutinen {PHA]) mitogen stimulation were also compared.
**Results**: The CD4+CD25+ regulatory cell populations examined in this study were demonstrated to have significantly higher expression of the FOXP3 marker compared with CD4+CD25- populations (p<0.001). At birth, there were no significant differences in the proportion of circulating regulatory cells, or allergen induced FOXP3 expression. However, by 6 months of age children who developed a subsequent allergic phenotype had significantly higher proportions of CD4+CD25+CTLA4+ regulatory cells (p=0.007), and higher allergen (ovalbumin) induced FOXP3 expression (p=0.02) compared with the nonallergic group. Although the allergic group tended to have higher TLR innate responses at birth (particularly for IL-6 [p=0.048] and IL-12 TLR4 responses), by 6 months of age the allergic group showed significantly lower IL-6 (p=0.039) and IL-12 (p=0.042) innate responses to the same TLR4 ligand (lipopolysaccaride). Consistent with previous reports, those with subsequent allergic disease had lower polyclonal responses to PHA at birth and at 6 months of age. At birth, this was most evident for IL-13 (p=0.019) and IFNγ (p=0.07), and at 6 months for IL-6 (p=0.012) and IL-10 (p=0.002).
**Conclusion:** Children who develop subsequent allergic disease show early differences in TLR mediated immune function and markers of regulatory T cell function. The pattern of these differences changes with age in the early postnatal period. Further studies are needed to determine the implications of this.

### 68
### Mechanisms of direct T cell suppression by IL-10 and TGF-beta

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IL-10 and TGF-β are key suppressor cytokines in T regulatory cells and antigen-presenting cells play essential roles in peripheral T cell tolerance to allergens, autoantigens, tumor antigens, transplantation antigens and chronicity in infections. Accordingly, the molecular pathways involved in direct T cell suppression by IL-10 and TGF-β have been investigated. Specific T cell activation requires T cell receptor (TCR) stimulation and the generation of co-stimulatory signals by the engagement of multiple cell surface receptors with their ligands. Major co-stimulatory signals are delivered to T cells by the interaction of CD2, CD28 and ICOS with LFA-3, CD80/CD86 and B7RP-1, respectively, which are essential for antigen-stimulated T cell proliferation and cytokine production. IL-10 directly inhibits CD2, CD28 and ICOS signaling in T cells through the src-homology-2 domain-containing tyrosine phosphatase (SHP-1). IL-10 receptor-associated tyrosine kinase Tyk-2 acts as a constitutive reservoir for SHP-1 in resting T cells and then tyrosine phosphorylates SHP-1 upon IL-10 binding. SHP-1 rapidly binds to CD28 and ICOS co-stimulatory receptors and dephosphorylates them within minutes. In consequence, the binding of phosphatidylinositol 3-kinase to either co-stimulatory receptor no longer occurs and downstream signaling is inhibited. The role of SHP-1 in IL-10-mediated suppression of CD2, CD28 and ICOS co-stimulations on T cells is demonstrated by using dominant-negative SHP-1 overexpressing T cells and silencing endogenous SHP-1 by small inhibitory RNA. Findings are confirmed using both SHP-1-deficient mice and IL-10-deficient mice. In conclusion, SHP-1-mediated inhibition of CD2, CD28 and ICOS signaling represents a novel mechanism for direct T cell suppression by IL-10. Similarly, TGF-β suppresses the CD2, CD28 and ICOS co-stimulations as well as it exerts a direct effect on T cell receptor signalling. TGF-β utilizes the transcription factors RUNX1 (runt-related transcription factor 1) and RUNX3, because their knock down by siRNA can abrogate TGF-β-mediated suppression. RUNX1 and RUNX3 are upregulated during the development of T regulatory cells by TGF-β. RUNX1 and RUNX3 bind and activate the gene expression via the FoxP3 promoter. These findings demonstrate novel molecular pathways in direct T cell suppression with IL-10 and TGF-β.

### 69
### Identification of IL-17RB+ NKT cells preferentially producing IL-13 as a novel subset responsible for development of AHR

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NKT cells are a population of autoreactive cells that mediate both protective and regulatory immune functions. NKT cells have so far been identified to be two subsets, a $CD4^+$ and a $CD4^-CD8^-$ (DN) population. Here, we identified a novel subpopulation of NKT cell specialized for induction of airway hypersensitivity reaction (AHR). The ARH-inducible NKT cells belong to CD4+ NKT cells expressing IL-17RB, one of the IL-17 receptor family members, whose ligand is IL-17E/IL-25, which is responsible for AHR. Among all leukocyte populations tested, about one-third of $CD4^+$ NKT cells, but not DN NKT cells were $IL\text{-}17RB^+$ cells. Moreover, $CD4^+IL\text{-}17RB^+$ NKT cells proliferate and produce predominantly IL-13, but not other Th2 cytokines (i.e., IL-5), in an IL-17E/IL-25 dependent manner and induce AHR, a cardinal feature of asthma. These results strongly suggest that $CD4^+IL\text{-}17RB^+$ NKT cells with unique and specialized phenotypes may have a crucial role, distinct from that of Th2 cells, in the induction of pathogenesis of asthma by bridging innate and Th2 immune responses.

## 70
### Clonal switch to IL-10-secreting type 1 T regulatory cells in high dose allergen exposure

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Healthy beekeepers, who receive multiple bee stings during bee keeping season represent a suitable model to investigate natural development and characteristics of antigen-specific T regulatory cells. Continuous exposure of bee keepers to high dose of venom antigens induces diminished T cell-related cutaneous late-phase swelling to bee stings in parallel to suppressed allergen-specific T cell proliferation and Th1 and Th2 cytokine secretion. After multiple bee stings, venom antigen-specific T cells show an *in vivo* clonal switch towards IL-10-secreting type 1 T regulatory (Tr1) cells within 7 days. T cell regulation continues as long as antigen exposure persists and returns to initial levels in a few months. Human $CD4^+$Th1 cells predominantly express HR1 and $CD4^+$Th2 cells HR2, which results in their differential regulation by histamine. Histamine induces the production of IL-10 by DC and enhances the suppressive activity of TGF-β on T cells. Histamine receptor 2 upregulated on specific T cells shows a dual effect by suppressing Th1 and Th2 cells and increasing IL-10 production. In addition, HR2 and IL-10 show synergic effects on the suppression of venom allergen-specific T cell proliferation via HR2. Apparently, these findings suggest that HR2 may represent an essential receptor that participates in peripheral tolerance to allergens. In addition to IL-10, CTLA-4 and PD-1 play roles in allergen-specific T cell suppression. In conclusion, the rapid generation capacity of allergen-specific Tr1 cells rather than their long life span and their use of multiple suppressor factors are decisive mechanisms of immune tolerance to allergens in healthy individuals. Rapid clonal switch to IL-10-producing Tr1 cells and upregulation of histamine receptor 2 during high dose antigen exposure represent mechanisms of peripheral tolerance to allergens in non-allergic individuals.

## 71
### A role for T cell independent interleukin 10 in allergen immunotherapy

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Human studies have suggested that allergen immunotherapy leads to the induction of regulatory immune responses that actively suppress the development of allergic inflammation. IL-10 production by allergen-specific T cells has been suggested as one of the main regulatory mechanisms. This was supported indirectly by in vivo observations in mice treated with IL-10 neutralizing mAb, in which the beneficial effects of immunotherapy were largely abrogated. However IL-10 is secreted by a variety of different cell types and the formal proof that T cell derived IL-10 is required for successful immunotherapy is still lacking.
In the present study we address this question using IL-10 null mutants ($IL\text{-}10^{-/-}$) and mice with a selective inactivation of the IL-10 gene in T cells generated by Cre/loxP-mediated conditional gene targeting. We used a protocol in which subcutaneous allergen immunotherapy is effectively suppresses allergen-induced allergic inflammation of the lung. Ovalbumin (ova) sensitized mice (wt, $Cre^+IL\text{-}10^{FL}$, $Cre^-IL\text{-}10^{FL}$, and $IL\text{-}10^{-/-}$) were treated with three subcutaneous ova injections on alternate days. One week later mice were challenged by ova inhalation and subsequently allergen specific antibody response, bronchoalveolar lavage and airway inflammation was analyzed. In addition, in vivo treatment with IL-10R blocking mAb was used in some of the groups.
Subcutaneous immunotherapy was effective in the suppression of allergen induced airway inflammation in control mice but not in IL-10 null mutants. Treatment with IL-10R blocking antibodies abrogated the protective effect of allergen immunotherapy in control mice. Interestingly, immunotherapy was also effective in $Cre^+$ $IL\text{-}10^{FL}$ mice with T cell-specific IL-10 deficiency and pretreatment with IL-10R blocking mAb partially reversed the protective effect of allergen immunotherapy. In conclusion, our data suggests that allergen immunotherapy is also effective in the absence of T cell derived IL-10, and that IL-10 from sources other than T cells contributes to the beneficial effect of subcutaneous immunotherapy.

## 72
### The “transistor model” of T cell differentiation

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The dichotomic Th1/Th2 paradigm has dominated our view on allergy pathogenesis and has produced important concepts such as the hygiene-hypothesis. Allergic immune responses are attributed to Th2 phenotype, acute neutrophilic responses are assumed to be Th17 dependent, late atopic skin reactions with Th1 cells and asymptomatic responses go along with regulatory T-cells. Although the Th1/Th2 paradigm is still a valid boundary for our immunologic concepts, it does not integrate the new T-cells subsets. Aim of the study was to analyze T-cell differentiation mechanisms of the new Th17 and $T_{reg}$-subsets to extend the Th1/Th2 paradigm or to formulate a new one.
We investigated the effects of transcription factors regulation of Th1, Th2 and Th17 using in vitro differentiation cultures driving naïve $CD4^+$ T cells towards effector or regulatory T cells. We used a novel protein-transduction method to demonstrate that we can directly interfere with gene regulation of the FOXP3 gene. Our data demonstrate that Th2 cells exclude $T_{reg}$ differentiation, while - against our previously formulated hypothesis - Th1 cells do not exclude $T_{reg}$-commitment. Interestingly, Th17 cells share pathways of $T_{reg}$ differentiation including the RORC2 transcription factor, which we identify here as TGF-β-inducible factor.
It is already known that T-bet inhibits Th17 differentiation and Th2 differentiation. Taken together it appears that transcription factors take the role of negative feedback regulators in the process of T-cell differentiation. Furthermore cytokines produced by the evolving T-cell subsets positively regulate their progenitors, creating a positive feedback loop. Therefore positive and negative loops regulate T-cell differentiation creating a regulative pattern of a transistor, as it is known for electronic networks. Transistors play a key role in information-integrating networks such as computers and may underlie the complex process of T cell differentiation. This “transistor-hypothesis” can now be used to compute our knowledge of T-cell differentiation in a system-biology approach supported by a starting algorithm and expand this algorithm to predict mechanisms controlling complex mechanisms such as allergen-tolerance.

## 73
### CD4+ T cells with regulatory characteristics and effector functions: A step beyond regulatory T cells

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# Abstracts

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We describe a subset of antigen-specific CD4+ regulatory T cells (Tregs) that does not produce either IL-10 or TGF-beta and is readily inducible in vivo. These cells are $CD25^{hi}$, $CTLA\text{-}4^{hi}$ and $ICOS^{hi}$, are anergic and are characterized by high expression of T-bet with constitutive lack of IL-2 transcription. Upon activation by MHC-class II dependent peptide interaction, and in the presence of IL-2, such cells induce apoptosis of dendritic or B cells through caspase activation, which results from combined action of Fas-FasL interaction and to some extent granzyme B secretion, but not of perforin. Bystander T cell activation is suppressed by a contact-dependent mechanism inducing apoptosis. In a model of experimental asthma, cytolytic Tregs migrate to lungs upon antigen exposure, prevent and suppress inflammatory cell infiltration as well as methacholine airway response. Adoptive transfer of transgenic B cells presenting the cognate antigen show that cytolytic Tregs eliminate antigen-presenting cells in vivo. Together with the description of a human Treg clone sharing the same properties, these data show that cytolytic CD4+ Tregs could represent a novel therapeutic approach for allergic and organ specific autoimmune diseases.

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### CD4+T cells from atopic dermatitis patients have differential expression of genes related to T cell homing proliferation and apoptosis mainly due to selective expression in the skin homing T cells

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**Background:** $CD4^+$ T cells play an important role in the pathogenesis of allergic inflammatory disorders, such as atopic dermatitis (AD), both systemically as well as in affected tissues.
**Aim:** To investigate the in vivo activation state of peripheral blood $CD4^+$ T cells of atopic dermatitis (AD) patients, by analyzing gene expression profiles of unstimulated $CD4^+$ T cells.
**Methods:** Unstimulated $CD4^+$ T cells from the PBMC of AD patients, allergic asthma (AA) patients and healthy controls (HC) were isolated by negative CD14 and positive CD4 immunomagnetic selection. MRNA, isolated from 5 AD and 7 HC, were analyzed using oligonucleotide arrays. Differentially regulated genes were validated by quantitative PCR (Q-PCR) in 8 AD, 8 AA, and 11 HC subjects. In addition, "typical" T helper type 1 (Th1)- and Th2-related genes were analyzed by Q-PCR. Of 8 AD patients and 5 HC the differential gene expression profile was determined in cutaneous lymphocyte-associated antigen $(CLA)^+$ and $CLA^-$ $CD4^+$ T cells
**Results:** The differential expression of 52 genes in AD patients was determined by microarray. Q-PCR confirmed several differentially regulated genes in AD. One group of genes, related to skin homing of T cells (CCR10, CCR4, CRTH2 and FUCT-VII), displayed increased expression. Another group of genes, related to apoptosis/proliferation (NR4A2, TNFAIP3, JUNB, C-JUN and GADD45A), displayed decreased expression. Remarkably, no genes directly related to Th2 or Th1 were found to be differentially expressed. When by Q-PCR the expression profiles were compared in the $CLA^+$ and $CLA^-$ population, similar expression of the skin homing related genes was found in the $CLA^+$ cells from AD patients and HC. However, the expression of the apoptosis/proliferation-related genes was significantly lower in the $CLA^+$ population of the AD patients compared to HC, but was comparable in both $CLA^-$ populations.
**Conclusion:** AD patients are characterized by increased numbers of $CLA^+CD4^+$T cells that directly affects the expression of skin homing genes in the total $CD4^+$ population. However, these $CLA^+CD4^+$T cells are qualitatively different from healthy controls by the decreased expression of apoptosis-related genes.

### 75

### Towards a better definition of the 'allergen-specific synapse': Molecular and functional analysis of the antigen receptor of Art v 1-specific helper T lymphocytes.

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T lymphocytes play a central role in the pathogenesis of allergic diseases. They critically contribute to the (early) sensitization phase as well as to late phase reactions in target organs. While major allergens, their immunodominant peptides and corresponding restriction elements have been characterized in detail during the past two decades the information on the molecular and functional definition of human allergen-specific T cell receptors (TCRs) remained scarce. Consequently, biological model systems based on molecularly defined human receptors and relevant allergen were still missing. Due to the uniform - mostly HLA-DRB1*01 driven - immune response against the sole immunodominant antigen in mugwort *(Artemisia vulgaris), i.e.* Art v $1_{25\text{-}36}$, allergy against mugwort appears to be an ideal model disease. Therefore we embarked on the cloning and functional characterization of a human αβ T-cell receptor (TCR) specific for Art v 1. TCR chains were RT-PCR-amplified from an Art v $1_{25\text{-}36}$-specific T-cell clone, retrovirally transferred and functionally tested in Jurkat T-cells or alternatively in PB T-lymphocytes of non-allergic individuals. The α-chain of the TCR is composed of TRAV17 and TRAJ45 segments, the β-chain uses TRBV18, TRBD1 and TRBJ2-7. Efficient TCR-transfer into Jurkat T-cells was shown by binding of TCR Vβ18-specific mAb and DRB1*0101/Art v 1 tetramers. Transgenic (tg) Jurkat T-cells specifically recognized syngeneic EBV B-cells pulsed with Art v $1_{25\text{-}36}$ peptide and artificial antigen presenting cells (aAPC) expressing invariant chain::Art v 1 fusion proteins. Moreover, transfer of the TCR into PBL generated T-cells that were Art v 1-reactive. Activation of tg T-cells by aAPC was strictly dependent upon co-stimulation. For the first time, a detailed molecular and functional analysis of a human allergen-specific TCR is presented. The availability of recombinant, allergen-specific TCRs will allow to set up standardized biological model systems in the future, in which all three components of the allergen-specific synapse (restriction element, immunodominant peptide, TCR) are defined at the molecular level and are based on a relevant human disease. Such systems shall provide important insights into the pathophysiology of allergic diseases and their possible cure in the future.

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### Chemokine Receptor Expression by Lung T cells in Asthma

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**Background:** Chemokines and their receptors could play key roles in the recruitment of $T_H2$ cells to the asthmatic lung. CCR8 is a chemokine receptor which is thought to be preferentially expressed on $T_H2$ cells. We have previously shown that although CCR3 and CCR4 are preferentially expressed on Th2 cells there was no increase in either the percentage of Th2 cells in BAL in mild asthma or in the percentage of CCR3 and CCR4 expressing T cells. We have now investigated the expression of the other Th2 associated chemokine receptor CCR8.
**Methods:** CCR8 expression was studied in blood and BAL T cells from asthma and normal subjects. CCR8 expression on IFN-$\gamma^+$ ($T_H1$) and IL-$4^+$/IL-$13^+$ ($T_H2$) cells was studied following stimulation with Phorbol-Myristate-Acetate and Calcium Ionophore.
**Results:** The percentage of CD3 T cells expressing CCR8 in blood was higher in severe asthma subjects (4.6%±0.6) compared to normal controls (3.3%±0.4; P=0.029). There was an approximately 6 fold enrichment of CCR8 on $T_H2$ cells compared to $T_H1$ cells (P<0.001) in both asthma and normal subject in both blood and BAL. Significantly more BAL T cells expressed CCR8 in asthma (8.6%±0.8) compared to normal subjects (3.9%±0.7) (p<0.01). In paired blood-BAL samples from asthmatics significantly more CCR8+CD3+ T cells were present in BAL (9.7%±1.1) than in blood (5.9%±1.2; P=0.045). There were more CCR8 positive

cells in bronchial biopsies from asthmatic (93±11 cells/mm$^2$) compared to normal subjects (30±16 cells/mm$^2$) (P<0.05). CCL1, the ligand for CCR8 was also increased in BAL from asthma subjects.
**Conclusion:** CCR8 is a good chemokine receptor marker of a $T_H2$ phenotype, and its expression is increased on asthmatic T cells, suggesting it could be involved in recruitment of $T_H2$ cells into the lung in asthma.

**77**
**Airways remodelling activity of the resistin-like molecule (RELM)-β) in asthmatic airways**

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RELM-β is a member of cysteine-rich cytokine family involved in insulin resistance and inflammation. RELM-β is expressed *in vitro* in human airway epithelial cells, fibroblast cells and smooth muscle cells. It was shown RELM-β enhanced airways inflammation and remodelling in a mouse model. However, its function in human asthma is uncertain. We hypothesized that RELM-β is elevated in asthmatic airways and plays a role in the pathogenesis of airways remodelling. Using the techniques of in situ hybridization, immunohistochemistry (IHC) and ELISA, we measured the expression RELM-β in bronchial biopsies and bronchoalveolar lavage fluid (BAL) obtained from asthmatics and controls (n=5 for each group), respectively. Effects of RELM-β on human lung fibroblast cell (MRC5) proliferation and differentiation, and on human endothelial cells *in vitro* was evaluated by MTT assay, Western blot and angiogenesis assay, respectively. Our results showed that expression of RELM-β, both at the mRNA and protein levels was increased in the asthmatic bronchial mucosa as well as BAL compared with controls. After 48 hours of stimulation, RELM-β significantly increased MRC-5 fibroblast cell proliferation and expression of α- smooth muscle actin (p<0.05). RELM-β also enhanced formation of microvessel structures by endothelial cells co-cultured with fibroblasts. Our data suggest that increased expression of RELM-β in asthmatic airways, especially in epithelial cells, may contribute to airways remodelling by inducing proliferation of airway fibroblast cells and differentiation of these cells into myofibroblasts, as well as angiogenesis.

**78**
**Mouse models for Kit mutations and mastocytosis**

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Mastocytosis is a heterogeneous disease characterized by expansion and accumulation of mast cells in different organs, particularly skin and bone marrow. Mast cell homeostasis depends on activation of stem cell factor/Kit signaling pathways and increasing evidence confirms the key role of activating somatic point mutations of *Kit* in the pathogenesis of mastocytosis. However, the relationship between the activating *Kit* mutations and the clinical heterogeneity is not well understood. To generate a mouse model for mastocytosis with controlled expression of the activating *KIT* mutation D814V (murine homolog of the typical human mutation D816V), a Bacterial Artificial Chromosome (BAC)-based transgene containing the entire *Kit* gene was introduced into the mouse genome. The transgenic *Kit* sequence contained the mutation D814V as well as a loxP-flanked transcriptional/translational „stop" cassette, allowing Cre-mediated induction of transgene expression. *Kit*$^{D814flox}$-transgenic were first bred to the hCMV-Cre line (del-Cre), which expresses Cre in all cell types resulting in expression of *Kit* D814V under the control of its endogenous regulatory elements. Most *Kit*$^{D814flox}$ del-Cre double transgenic mice died perinatally and showed strongly induced hematopoiesis associated with an increase of erythroid progenitors. Few *Kit*$^{D814flox}$ del-Cre transgenic mice survived for several months, but developed mast cell tumors and cutaneous mastocytosis. Breeding of *Kit*$^{D814flox}$ mice to the vav-Cre line expressing Cre exclusively in the hematopoietic system resulted in a comparable phenotype confirming that alterations within the hematopoetic system are responsible for the observed phenotype. Next, *Kit*$^{D814flox}$ mice were crossed to the inducible Mx1-Cre line that expresses Cre upon induction by type I interferon. About three months after induction, *Kit*$^{D814flox}$ Mx1-Cre mice developed mast cell tumors and mastocytosis, sometimes also associated with hematologic neoplasia. To further limit expression of *Kit* D814V, we are currently breeding the *Kit*$^{D814flox}$ line to a newly generated mast cell-specific Cre line. Our new mouse models will allow insights into the pathogenesis of mastocytosis and the function of mutated *Kit* in various cell types. They will also be useful for analyzing Kit-targeted therapies.

**79**
**IL-31 receptor alpha expression in epidermal keratinocytes is modulated by cell differentiation**

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Recently, interleukin (IL)-31 has been identified as a 4-helix bundle cytokine that is expressed by activated T cells preferentially by T-cells skewed toward a TH2-type cytokine profile. So far, biological functions of this novel cytokine were mainly analyzed in skin diseases such as atopic dermatitis or allergic contact dermatitis. While an enhanced expression of IL-31Rα has been described in inflammatory skin diseases, the influence of cell differentiation on the expression of the IL-31 receptor in skin cells was largely unknown. Therefore we analyzed the effects of the cellular differentiation state on epidermal mRNA and protein levels of IL-31Rα in culture conditions when epidermal cultures exhibit predominantly basal, spinous, and granular cell phenotypes. The differentiated keratinocyte phenotype was defined by cellular morphology and expression of differentiation-specific gene markers. Interestingly, IL-31Rα mRNA completely disappeared during the process of differentiation and already at the transition from basal to spinous phenotype (d5) hardly any mRNA was detectable by quantitative RT-PCR. Expression of OSMRβ mRNA was decreased on day 5 but not significantly altered during the late phases of keratinocyte differentiation. Using an IL-31Rα–specific antibody we confirmed a strong down-regulation of the receptor at the protein level when differentiation of NHEK was induced by culturing the cells in growth medium containing 1.4 mM $Ca^{2+}$ for more than 3 days. Protein expression was not traceable in terminal differentiated keratinocytes (day 15) resembling a granular cell phenotype. Additionally, we found no IL-31Rα expression in dermal fibroblasts or primary human melanocytes. Monocytes stimulated with 1000 U/ml IFNγ for 48h revealed a strong upregulation of IL-31Rα expression. Loss of receptor expression in late-stage keratinocytes consequently led to a loss of cytokine responsiveness: while proliferating keratinocytes respond to IL-31 treatment with increased tyrosine phosphorylation of STAT3, we could not detect any STAT activation in differentiated keratinocytes. In contrast, stimulation with OSM resulted in STAT3 tyrosine phosphorylation irrespective of the differentiation state which indicates that differentiated keratinocytes do respond to cytokine treatment in general. Therefore *in vitro* studies analyzing the effects of the novel cytokine IL-31 on epidermal keratinocytes should consider the variation of IL-31Rα expression which depends on the status of cellular differentiation.

**80**
**Comparison between human neutrophils and eosinophils in matrix metalloproteinase-9 release**

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**Background:** Proteinases such as matrix metalloproteinase-9 (MMP-9) play important roles in the pathogenesis of bronchial asthma and chronic obstructive pulmonary disease (COPD). However, the main source of proteinases and the mechanism of proteinase release have not been thoroughly elucidated. In this study, we examined MMP-9 release using soluble agonists and compared its release between human neutrophils and eosinophils. **Methods**: Neutrophils and eosinophils isolated from human leukocytes were incubated with soluble agonists such as FMLP for 20 min. In some experiments, cytochalasin B or GM-CSF was added 10 min before stimulus addition. At the end of the incubation, MMP-9 in supernatants was measured by ELISA. **Results:** Without stimulus, neutrophils released significant amounts of MMP-9 spontaneously compared to eosinophils. FMLP clearly enhanced MMP-9 release from neutrophils, but did not affect eosinophils. Eosinophils released no significant amount of MMP-9 in response to

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other stimuli, C5a or PAF, even if with IL-5 priming. GM-CSF clearly enhanced FMLP-induced MMP-9 release. With cytochalasin B pretreatment, neutrophils did not release significant amount of MMP-9 in response to FMLP compared to the control. Pretreatment of neutrophils with pertussis toxin resulted in the inhibition of FMLP-induced MMP-9 release, indicating the contribution of PTX-sensitive G-proteins to intracellular signal transduction in FMLP-induced MMP-9 release.
**Conclusion:** These results suggest that neutrophils release large amounts of MMP-9 in response to FMLP, which is a bacterial product analogue. It is possible that MMP-9 released from neutrophils, not from eosinophils, may play roles in the pathogenesis of bronchial asthma and COPD.

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### Protease-Activated Receptor-2 (PAR-2) activation mediates allergic sensitization to cockroach extracts

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**Introduction**: A number of common aeroallergens, such as cockroach house dust mite and fungal allergens, have serine protease activity which is important for allergic sensitization. Cockroach allergens are very common in urban environments and they are associated with increases in the incidence and severity of asthma. Cockroach extracts contain serine protease activity and some of these proteases activate Protease-Activated Receptor-2 (PAR-2). PAR-2 is activated by serine proteases, including some of the aeroallergens mentioned above, and has been implicated in inflammatory reactions. We have shown that PAR-2 activation leads to allergic sensitization to concomitantly administered antigens implicating this receptor in the pathogenesis of asthma.
**Hypothesis:** PAR-2 activation in the airways by natural allergens with serine protease activity is important for the development of allergic sensitization. To test the hypothesis we used a murine model of mucosal sensitization to cockroach extracts.
**Methods:** For allergic sensitization, cockroach extract was administered i.n. daily for 5 days. Mice were later challenged with cockroach extract for 4 consecutive days and then allergic airway inflammation and airway hyperresponsiveness (AHR) were assessed. To study the role of PAR-2, mice were administered a blocking anti-PAR-2 antibody i.n. before each cockroach administration during the sensitization phase.
**Results:** Mice that were sensitized and challenged intranasally with cockroach extract developed eosinophilic inflammation and AHR. AHR was as high as that seen in mice sensitized to cockroach following an intraperitoneal injection with aluminum hydroxide, but airway inflammation was lower. Allergic sensitization after intranasal administration of cockroach extract was also present in TLR-4 knock out mice indicating that LPS did not mediate allergic sensitization. Administration of an anti-PAR-2 blocking antibody during the sensitization phase completely inhibited the development of AHR and airway inflammation to cockroach extracts. Cockroach extract administration induced altered dendritic cell migration to lymph nodes and altered dendritic cell maturation.
**Conclusions:** Mucosal exposure to cockroach extract induces PAR-2-dependent allergic airway sensitization. This mechanism may be used by all allergens with serine protease activity to induce allergic sensitization.

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### Expression and cellular provenance of thymic stromal lymphopoietin (TSLP) and chemokines in patients with severe asthma, COPD and controls

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Asthma and chronic obstructive pulmonary disease (COPD) are suggested to result from Th2-type and Th1-type airway inflammation respectively. Thymic stromal lymphopoietin (TSLP) favours Th2 type inflammation. We hypothesised that airways expression of TSLP and Th2-attracting chemokines is increased in asthma, but not COPD, where Th1-attracting chemokines predominate. We used *in situ* hybridization and immunohistochemistry to examine the expression and cellular provenance of TSLP, Th2-attracting (TARC/CCL17, MDC/CCL22, and I-309/CCL1) and Th1-attracting (IP-10/CXCL10 and TAC/CXCL11) chemokines in bronchial biopsies from 13 patients with severe asthma, 15 with COPD (ex- and smokers) and 30 normal controls (non-smoker, ex-smoker and smoker, 10 for each group). The results showed that the numbers of cells within the bronchial epithelium and submucosa expressing mRNA for TSLP, TARC/CCL17, MDC/CCL22 and IP-10/CXCL10, but not I-TAC/CXCL11 and I-309/CCL1, were significantly increased in severe asthma and COPD as compared with non-smoker controls. Expression of these molecules was also increased in ex- and smokers as compared with normal non-smokers. TSLP and TARC/CCL17 expression correlated inversely with airways obstruction in asthma and COPD. Sequential IHC/ISH showed that epithelial cells, endothelial cells, neutrophils, macrophages and mast cells were the sources of TSLP and Th1 and Th2 attracting chemokines. The cellular provenance of these mediators was strikingly similar in severe asthma and COPD. Our data implicate TSLP and both Th1- and Th2-attracting chemokines in the pathogenesis of asthma and COPD, and provide evidence for uniformity in the origins and expression of these mediators, particularly in the epithelium, in obstructive airways disease.

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### IgE against Staphylococcus aureus enterotoxins: Relevance to disease expression and severity in asthma

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**Background:** Asthma is a complex syndrome with a spectrum from mild intermittent to severe persistent disease. The basis for severe persistent disease is poorly understood. We have investigated the association and relevance of specific IgE against Staphylococcus *aureus* enterotoxins (SEs) to asthma and its severity.
**Methods:** Serum specific IgE against SEs were measured in 49 non-asthmatic volunteers (control subjects) and 107 volunteers with established asthma. Based on their treatment requirements, these asthmatics comprised 55 with mild intermittent disease and 52 with severe persistent disease. To verify the findings a further study was conducted in 76 steroid controlled asthmatics (SCA) and 93 severe asthmatics (SA) recruited by the ENFUMOSA study group. To understand the relevance of the findings, additional bronchoscopic studies evaluated the presence of Staphylococcus *aureus* within airway luminal samples and the presence of SE specific IgE in bronchial tissue samples.
**Results:** Serum concentrations of SE specific IgE were significantly greater in asthma than non-asthma ($p<0.0001$). The percentage positive in each group was significantly lower in the controls (16.3%) than in mild asthma (34.5%, $p<0.05$) or severe asthma (53.8%, $p<0.0001$), with a significant difference between the two asthma groups ($p<0.001$). These findings contrasted with indoor aeroallergen sensitivity, with such sensitisation in the mild asthmatics (74.1%) being significantly greater than in either the non-asthmatic controls (34.7%, $p<0.001$) or severe asthmatics (37.2%, $p<0.001$). The second study revealed high percentages of individuals with elevated levels of SE IgE in both SCA (43.7%) and SA (50.6%). There was a significant relationship between the group % SEIgE +ve and the group mean $\%FEV_1$ in the 4 asthma groups ($r=0.952$, $p<0.048$), with declining lung function with increasing positivity. PNA-FISH revealed S*taphylococcus aureus* more frequently in bronchoalveolar lavage cytospins from severe asthma than controls and to a greater extent ($p<0.03$), whilst specific SE IgE was only evident in the bronchial mucosa of severe asthma and not healthy controls.
**Conclusions:** These findings identify the relevance of bacterial superantigens to the pathogenesis of asthma, in particular severe persistent asthma, and indicate that asthma may be driven by IgE-associated mechanisms despite the absence of aeroallergen sensitisation.

**84**

**Airway allergen exposure during respiratory viral infections can induce asthma by both Th1 and Th2 immune responses**

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Viral infection during the first 3 years of life greatly enhances the risk of developing asthma in children, and experimental data in animal experiments support this idea. Although respiratory viral infections in early childhood may enhance the development of airway allergen sensitization, the exact mechanisms of the effects of viral infections on the adaptive immune response to inhaled allergens are controversial. Respiratory viruses produce double-stranded (ds) RNA during their replicative cycles that stimulates an innate immune response and modulates subsequent adaptive immune responses.

To evaluate the effects of dsRNA on airway sensitization to inhaled allergens, we made novel mouse models that were created through airway simultaneous sensitization to an allergen and low or high doses of dsRNA. To evaluate underlying pathophysiologic and signaling mechanisms in the development of allergic lung inflammation, the mouse models were applied to TLR3-, IL-13-, IL-4-, STAT6-, IFN-gamma-, STAT-4-, and T-bet-deficient mice.

We found that airway allergen sensitization with dsRNA induced lung inflammation and airway hyperresponsiveness (AHR) that was not developed in TLR3-deficient mice. Moreover, lung inflammation and AHR enhanced by low dose dsRNA was impaired in IL-13-deficient mice, whereas lung inflammation and AHR by high dose dsRNA was impaired in IFN-gamma-deficient mice. The models also demonstrated that low dose dsRNA enhanced IL-4 expression during allergen sensitization and that inflammation enhanced by low dose dsRNA was not developed in IL-4- or STAT6-deficient mice. In contrast, the present study showed that high dose dsRNA enhanced IFN-gamma expression and IL-12 during allergen sensitization and that the development of lung inflammation enhanced by high dose dsRNA was impaired in STAT4- or T-bet-deficient mice.

These findings suggest that airway allergen exposure during respiratory viral infections can induce asthma by both Th1 and Th2 immune responses.

**85**

***In vitro* corticosteroid treatment enhances TNF-α-mediated inflammatory reactions of pulmonary microvascular endothelial cells**

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**Background:** TNF-α is a major proinflammatory cytokine, which is thought to be important in the pathogenesis of inflammatory diseases such as asthma. A major site of TNF-α action is the vascular endothelium, where it enhances inflammatory responses by regulating leukocyte extravasation into inflamed tissue.
**Objective:** In order to investigate the effects of dexamethasone (DEX) on the TNF-α-mediated responses by human lung blood microvascular endothelial cells (HMVEC-LBl) *in vitro*.
**Methods:** HMVEC-LBl were cultured with TNF-α in the presence or absence of DEX. Chemokine expressions were determined by real-time PCR and ELISA. Cell surface expressions of VCAM-1 and ICAM-1 were analyzed by flow cytometry. Leukocyte adhesion assay was performed using calcein-AM labeled human peripheral eosinophils and neutrophils. Apoptotic cells were determined by annexin V and PI staining.
**Results:** TNF-α markedly induced CXCL1, CXCL8, CXCL10 and CCL5 productions and cell surface expressions of ICAM-1 and VCAM-1 on HMVEC-LBl. TNF-α-induced CXCL1 and CXCL8 were slightly but significantly attenuated by DEX treatment. In contrast, TNF-α-induced CXCL10, ICAM-1 and VCAM-1 were significantly enhanced by DEX up to approximately 150%. Correspondingly, *in vitro* adhesion of eosinophils and neutrophils to TNF-α-stimulated HMVEC-LBl were significantly enhanced by DEX. TNF-α-induced apoptosis of HMVEC-LBl was blocked by DEX.
**Conclusions:** Corticosteroid was found to enhance TNF-α-mediated *in vitro* expressions of CXCL10, ICAM-1 and VCAM-1, and blocked TNF-α-induced apoptosis of HMVEC-LBl. It should be further studied in future whether TNF-α-mediated extravasation of leukocytes and survival of endothelial cells are upregulated by corticosteroids therapy *in vivo*. Nevertheless, our data suggested that corticosteroids should be given with a caution for TNF-α-mediated vasculitis or hypervascularity that are often seen in intractable asthma.

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**Nitric Oxide And Ceramide Pathway Interaction: A New Target In Allergic Asthma**

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Reactive oxygen and nitrogen species (ROS and RNS respectively) are environmental pollutants affecting lung epithelial cell functions by modulating inflammation, cell proliferation, growth or apoptosis. Because ceramide is a second messenger molecule modulating cell apoptosis and oxidative stress, we hypothesized that ceramide *upregulation* contributes to airway hyperreactivity and inflammation during asthma. The availability of ceramide is fine-tuned by the rate of generation involving sphingomyelinases, the *de novo* synthesis from sphingosine and the rate of degradation catalyzed by ceramidases. Therefore a number of pathways can account for altered ceramide levels in pathophysiological situation.
In models of allergic bronchospasm in actively sensitized guinea-pigs, the aerosol administration of the antigen determined an increase in ceramide levels in the airway epithelium. Ceramide increased concurrently with markers of oxidative stress (3-nitrotyrosine, PARP and 8-OHdG) and apoptosis (caspase 3 activity), and was associated with a profound deactivation of MnSOD in lung tissues.
In addition, ceramide *upregulation* was associated with the development of an inflammatory response characterized by eosinophil and neutrophil infiltration in the lung tissue, as well as elevation of prostaglandin $D_2$ and proinflammatory cytokines in bronchoalveolar lavage. These effects were associated with bronchial hyperreactivity. The treatment of the animals with nitric oxide (NO) donors increased cellular ceramide levels without any apoptotic response. Inhibition of the enzymes controlling *de novo* ceramide synthesis attenuated nitrotyrosine formation and oxidative/nitrosative stress, epithelial cell apoptosis and airway inflammation while improving the development of respiratory and histopatological abnormalities. These results implicate ceramide in the development of allergic airway inflammation and hyperresponsiveness and suggest that sphingomyelin/ceramide pathway could be a novel therapeutic target for inflammation and airway hyperreactivity in allergic asthma.

**87**

**Interactions between Toll like receptor-3 (TLR-3) and IgE dependent pathways in human lung tissue explants**

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TLR-3 recognises dsRNA and is an important component of the innate response to viral infection. We have examined the effects of the synthetic TLR-3 ligand, poly I:C, on cytokine release from human lung parenchymal explants and examined the effects of a subsequent exposure to anti-IgE. We examined the release of a range of cytokines, lymphokines and chemokines.

Human parenchymal explants (n=18) were incubated in either a buffer control or 100µg/ml poly I:C for 1 hour and then stimulated with either buffer, 100µg/ml anti-IgE or 100ng/ml LPS as a positive control. Tissue was removed 24 hrs later, weighed and stored at –70°C and the supernatant aliquotted and stored at –70°C. Cytokines were measured by ELISA and corrected for tissue weight.

Immunohistochemistry demonstrated that human parenchymal tissue contained substantial numbers of mast cells with FcεRI as well as TLR3 positive cells that included macrophages and mast cells.

# Abstracts

Tissue stimulated with poly I:C released little or no TNFα or IL-1β, significant levels of the lymophokine IL-10 and the chemokine MIP-1β (CCL4) but failed to release IL-8 (CXCL8) consistently. Finally, poly I:C stimulated the release of the Th2 cytokine IL-13 see table 1. Anti-IgE caused the release of significant levels of all of the cytokines..

| Cytokine | Buffer control | Poly IC (100µg/ml) | Anti-IgE (100µg/ml) | Poly I:C + Anti-IgE |
|---|---|---|---|---|
| TNFα (pg/mg tissue) | 1.9±0.9 | 10.7±3.8* | 32.4±12.4* | 43.2±17.8 |
| IL-1β (pg/mg tissue) | 1.4±0.4 | 1.9±0.5 | 8.6±2.6* | 7.8±2.2 |
| IL-10 (pg/mg tissue) | 0.4±0.1 | 6.9±2.6* | 4.6±1.0* | 6.1±1.2 |
| MIP-1β (pg/mg tissue) | 16.8±3.8 | 95.5±22.6* | 194.7±76.8* | 248.9±91.0 |
| IL-8 (ng/mg tissue) | 2.4±0.6 | 3.5±1.0 | 5.8±1.5* | 7.4±2.1 |
| IL-13 (pg/mg tissue) | 4.0±0.5 | 8.6±3.0* | 7.9±1.6* | 8.1±2.8 |

**Table 1** Cytokine release from human lung parenchymal explants. *indicates P<0.05 compared to buffer control

The combination of polyI:C and anti-IgE failed to increase cytokine release above the levels seen with anti-IgE alone in any of the cytokines tested.

In summary our experiments show that poly I:C can initiate the release of cytokines from human lung parenchymal tissue but despite the presence of TLR-3 on human lung mast cells there is no evidence of synergy between TLR-3 and IgE dependent pathways.

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### The nasal mucosa is an important site for induction, maintenance and therapy of systemic allergy

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The nose is an important target organ in type I allergy, and is usually the place of first contact with respiratory allergens. We have investigated the role of the nasal mucosa as a barrier against allergens as well as in the control of systemic allergen-specific antibody levels and sensitivity.

We used an *in-vitro* model of the respiratory mucosa to analyse the influence of several factors and mediators on epithelial permeability for allergens. Cigarette smoke exposure, rhinovirus infection, and the Th1-derived cytokine interferon-gamma were shown to impair the barrier function of the mucosa. The resulting increased influx of allergens led to enhanced effector cell activation and may thus enhance allergic inflammation. The role of the nasal mucosa in the regulation of IgE levels and systemic sensitivity was investigated using controlled nasal provocation experiments and by following pollen-allergic patients during seasonal allergen exposure. Nasal but not dermal allergen contact induced a strong boost of serum IgE levels and systemic sensitivity to allergens. The latter could be attributed to a more efficient loading of effector cells with allergen-specific IgE antibodies.

We also searched for factors which are able to reduce the allergen exposure-induced increase of specific IgE. The effects of nasal corticosteroids on allergen-induced rises of specific IgE were studied in a double-blind, placebo-controlled clinical study. We demonstrated that nasal fluticasone did not inhibit but rather increased the rise of allergen-specific IgE levels following nasal provocation with recombinant allergens. In another approach we have shown that therapy with genetically modified Bet v 1 derivatives reduced seasonal boosts of Bet v 1-specific IgE-levels. An analysis of antibody responses in vaccinated patients revealed that this therapy induced $IgG_1$ and $IgG_4$ antibody responses in sera and nasal secretions which were directed against new, sequential epitopes overlapping with IgE epitopes. These antibodies were able to inhibit allergen-induced basophil degranulation and also may have prevented allergen-induced boosts of IgE production.

In summary, our results give evidence that the nasal mucosa is not only a target organ of allergy but also an important site for the regulation of systemic IgE levels and sensitivity.

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### Induction of allergic airway inflammation by house dust mite allergen specific Th2 cells in mice

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**Aim:** It is known that allergic inflammatory diseases such as asthma are Th2 cells-mediated, however, the pivotal roles of allergen-specific Th2 cells in the induction of allergic lung inflammation have not been fully elucidated. The aim was to elucidate the imunopathological roles of allergen – specific-Th2 cells in allergic airway inflammation.
**Methods**: Mice were epicutaneously sensitized with a major dust mite allergen, Blo t 5 and a well-characterized Blot -5 specific-Th2 cell line was subsequently established from the splenocytes of the sensitized mice. The immunopathological roles of the cell line were assessed *in vivo* by adoptive cell transfer approach. .Naïve mice received Blo t 5 specific-Th2 cells intravenously followed by intranasal challenge with Blo t 5. The responses of recipient mice were analysed by immunological and histochemical methods.
**Result:** A long term $TCRV\beta3^+$ Blo t 5 specific Th2 cell line producing high levels of IL-4, IL-5, IL-13 and IL-10 but not IFN-γ was established. These $CD44^{high}CD62L^-$ Th2 cells showed up-regulation of CTLA-4, ICOS, OX40, 4-1BB, CD27 but not CD40L upon stimulating with Blo t 5. After intranasal challenge with Blo t 5, Th2 cells recipient mice developed Blo t 5-specific IgG1 and IgE, airways eosinophilia and mucus production of the Goblet cells. In addition to the donor Th2 cells, the cellular infiltrate consisted of $CD4^+$, $CD8^+$ T cells and NK cells of the recipient mice. Such cellular inflammation could be suppressed by dexamethasone intervention. The pathological results were not observed in the PBS challenged recipient mice.
**Conclusion**: Blo t 5-specific Th2 cells played a central pathological role in mediating allergic airway inflammatory responses resembling those seen in humans. This animal model is particularly useful for screening of novel therapeutics for asthma and allergy.

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### Regulation of intraepithelial accumulation of mast cells in the nasal mucosa of allergic rhinitics

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**Background:** Allergic rhinitis (AR) is characterized by the intraepithelial accumulation of mast cells. Yet, the precise mechanisms are not well defined. SCF has been considered to play a pivotal role, but we previously reported that nasal mast cells (NMC) express CCR3 and exhibit increased chemotaxis to RANTES, suggesting a role for RANTES in mast cell migration.
**Purpose:** In order to precisely elucidate the factors regulating mast cell migration into the allergic nasal epithelium, we analyzed the levels of RANTES, Eotaxin and SCF in the epithelium and lamina propria (LP) of patients with AR and the kinetics of RANTES+, tryptase+, and CCR3+ cells in the epithelium and LP after nasal allergen challenge (NAC) in patients with AR.
**Methods:** By ELISA, we analyzed the levels of RANTES, Eotaxin and SCF

in homogenized nasal scrapings and deep lamina propria of AR patients. We performed NAC with mite antigen in patients with AR and took biopsies at 30 min, 6 hrs, and 12 hrs post NAC. By immunohistochemisty, we analyzed the expression of RANTES, Eotaxin and SCF in the epithelium and LP, and the number of tryptase+ RANTES+, eotaxin+ and CCR3+ cells in challenged and control biopsies.
**Results:** The level of RANTES, but not Eotaxin and SCF was greater in the nasal scrapings (epithelium) than in the deep LP. Immunoreactivity for RANTES was marked in the epithelium as compared to that of SCF or Eeotaxin. At 30 minutes post NAC, tryptase+, RANTES+ and CCR3+ cells were increased in the epithelium. At 6 hours post NAC, tryptase+ cells and RANTES+ cells were still increased in the epithelium but at 12 hrs post NAC only tryptase+ cells were increased. Intraepithelial migration of mast cells occurred as early as 30 min and. paralleled the increase in CCR3+ and RANTES+ cells. Furthermore, mast cell mediators histamine and tryptase upregulated RANTES production from nasal epithelial cells.
**Conclusions:** Taking together our findings on the increased chemotaxis of mast cells to RANTES, the CCR3 expression on NMC and the present results, RANTES may be a critical factor regulating mast migration into the allergic nasal epithelium.

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### Non-IgE mediated chronic allergic skin inflammation revealed with rBet v 1 fragments

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder affecting about 3-10% of patients suffering from IgE-mediated allergies. However, the contribution of IgE to chronic allergic inflammation is a matter of discussion. We have used the major birch pollen allergen, Bet v 1 and two non-IgE-reactive recombinant Bet v 1 fragments to dissect the contribution of IgE-versus non-IgE-mediated effects in chronic allergic inflammation. IgE reactivity to rBet v 1 and rBet v 1 fragments was tested in a dot blot assay and confirmed by skin prick testing. Birch pollen allergic AD patients showed immediate type skin reactions to rBet v 1 but not to equimolar mixes of the rBet v 1 fragments. When used for atopy patch testing, non-IgE-reactive rBet v 1 fragment mix induced a positive eczematous reaction in almost all birch pollen allergic AD patients. Our finding that Bet v 1 fragments containing only T cell epitopes but no IgE epitopes could induce chronic allergic skin inflammation provides evidence for an important role of non-IgE-mediated mechanisms in chronic allergic skin inflammation in AD patients.

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### The Use of Recombinant Allergens Provides Improved Solutions for Patients with Insect Venom Allergy

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The clinical approach to diagnosis and treatment of venom allergy is based on the use of natural extracts. Venom SIT with natural extracts is effective in the majority of allergic patients, but SIT failures and side effects as well as diagnostic problems with extracts have been reported in 10-40%. Thus, there is considerable interest in improving diagnostic and immunotherapeutic procedures in venom allergy. The use of recombinant allergens may provide such an improvement, but only a limited number of venom allergens are available so far as recombinant proteins. The aim of our study was to (**i**) identify, characterize and express those venom allergens which were still elusive (e.g., Api m 3, Api m 5), and (**ii**) provide a panel of the most prominent allergens from *A. mellifera* and *V. vulgaris* for analyzing sIgE reactivity in venom-allergic patients.
Api m 1, Api m 2, Api m 3, Api m 5, Api m 6, and Api m 7, as well as Ves v 2, Ves v 5, and a 100 kD vespid allergen were cloned from venom gland cDNA, identified by sIgE, expressed in insect cells, and used for characterization and immunoreactivity studies.
Api m 5 exhibits a homology of 32% to a human dipeptidyl peptidase IV (DPPIV). Purified rApi m 5 is specifically recognized by sIgE from honeybee venom allergic patients (48%; n=54). All other expressed venom allergens bound sIgE to various extents (25-90%, n=88). Together with nApi m 1, and rApi m 2, rApi m 3 and rApi m 5 were used in a pilot component-resolved approach in patients who had been negative with bee venom extract (sIgE<0.35 kU/L) despite a history of ≥grade II anaphylaxis. Surprisingly, no or rare sIgE reactivity was found with the abundant allergens Api m 1 (0/8), Api m 5 (1/8) and Api m 2 (2/8), while most of the sera were found to be reactive with Api m 3 (7/8). Such a differential sIgE reactivity was not found in an otherwise identical control group characterized by a positive sIgE test result for bee venom: Api m 1 (10/12), Api m 2 (6/12), Api m 3 (10/12), Api m 5 (8/12). These results indicate a critical importance of sIgE reactivity to Api m 3 not reflected in natural extracts. Next, we analyzed sera from honeybee venom SIT failures (n=10) for sIgE reactivity with Api m 1-3, and 5. Sera from successfully treated patients were used as control (n=25). In 6/10 SIT failure patients, either an increase in sIgE or a *de-novo* sIgE sensitization to Api m 3 was observed, while none of the successfully treated patients showed this feature.
In summary, the expression in insect cells appears to be a suitable approach for the production of recombinant hymenoptera venom allergens, thus providing valuable tools for the development of component-resolved diagnostic approaches as well as the basis for safer and more efficacious venom SIT.

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### Microarray Of Alllergenic Components-based Diagnosis In Polysensitized Patients

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The aim of this study was to analyze the sensitization profile to allergenic components of polysensitized allergic patients diagnosed previously using standard techniques defined as two or more sensitizations to different allergenic sources.

Plasma from 55 atopic polysensitized patients was used to measure semi-quantitatively specific immunoglobulin E (IgE). In 20 patients, specific IgE against 79 allergenic component, and in 35 patients specific IgE against 85 allergenic component were tested using the microarray ISAC CRD 79 and 85 (VBC-Genomics, Austria), respectively.

Patients were classified into 3 different categories: *genuine sensitization*, *mixed sensitization* and *panallergens sensitization*. 32 patients showed *genuine sensitization* as sensitizated to majoritary allergens without sensitization to panallergens (50% Phl p 1 and Phlp p 5; 50% Der p 1 and Der f 1). 19 patients showed *mixed sensitization* both to majoritary allergens and panallergens (13 patients sensitizated to Phl p 1 and Phlp p 5 and all profillins tested; 2 patients sensitizated to Phl p 1 and Phlp p 5 and polcalcin Phl p 7; and 4 patients sensitazed to Der p 1, Der f 1 and tropomyosin). Finally, 4 patients showed *sensitization to panallergens* such as profillins (1 patients) without sensitization to majoritary allergens of grasses neither to tree pollens and tropomyosins (4 patients) without sensitization to majoritary allergens of dust mites.

In addition, we observed that 30% of the patients were sensitized to all the profillins tested and 28% to any of them. Moreover 18% of the patients showing sensitization to any allergen of dust mites were sensitizated exclusively to group 2. All the tropomyosins tested in the microarray (Der p 10, Per a 7, Ani s 3, Pen i 1 and Pen m 1) show the same reactivity pattern suggesting the homology between all tropomyosins.

In conclusion, diagnosis based in molecular components applied to allergy is a practical tool for polysensitized patients and could change diagnosis of sensitizations modifying therapeutical management in the future.

# Abstracts

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**Utility of a new solid phase multiplex technology to determine patient sIgE responses to whole milk extracts and specific allergenic proteins simultaneously.**

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The study set out to demonstrate as a proof of concept that the OPTIGEN® system can be used to determine sIgE reactions to whole extracts and specific allergenic proteins within the extract simultaneously.

The technology was developed for the multiplex determination of sIgE to up to 36 different allergens in a panel format in one device. For this study, whole milk extract, casein, alpha casein, beta casein, kappa casein, alpha lactalbumin Type I, beta lactoglobulin (A and B), serum albumin and lactoferrin were bound to the OPTIGEN® solid phase. Milk positive and negative patient samples were tested and the results were compared to Phadia CAP®.

A total of 48 results were available, of which 40 agreed between OPTIGEN® and CAP®. Eight results where discrepant between the two methods, of those, 5 were a class 0 – 1 discrepancy. Therefore if those are removed as low-end variation there are 3 true discrepant results, which could be due to differences in allergen composition, solid phase or interfering substances in the serum sample. The % agreement is then 93% between the two methods.

This study demonstrates that OPITGEN® can be used as a technology to test for sIgE reactions to whole extract and specific allergenic proteins simultaneously thus providing greater resolution and additional diagnostic value that can be missed when the whole extract is used. The advantage of using this method versus single allergen tests being that this is a proven multiplex technology for IgE determination, utilizes low volume of sample (<300ul) and very low volumes of allergen, is easy to use since the whole extract and specific proteins are pre-coated in one device, is low cost and results can be obtained in less than 5 hours.

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**Identification and characterization of lentil sensitization patterns**

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**Introduction:** The legume lentil is common food in parts of the (sub) tropical world because of its high nutritional value and it found its way into food preparations in the western industrialized society. This may subsequently induce lentil specific allergies but also evoke symptoms in other established legume sensitized patients as a consequence of crossreactive IgE.
**Objective:** It was our aim to identify and characterize (crossreactive) lentil sensitization patterns using serum from lentil sensitized patients from 3 different countries plus the EuroPrevall serum panel [1)].
**Material and Methods:** Serum from lentil sensitized patients was used to detect lentil allergens. In order to detect heat resistant allergens, boiled lentil extract was monitored for IgE binding on SDS/PAGE-immunoblot. Lentil allergen was partially purified by size exclusion chromatography (SEC). Further column fractionation followed by MS must identify which allergens are present. This enables us to study purified allergens with respect to their clinical relevance/immunochemical properties. CAP- and blot-inhibiton experiments with other legumes are necessary to detect crossreative structures.
**Results:** SDS/PAGE showed major protein bands, indicating the presence of several storage proteins. Immunoblotting with serum from Spain, UK and the Netherlands showed major IgE binding proteins at 50 and 64 kdA, presumably Len c 1 and 2. Other bands were observed at 40 and 55 kDa. Repeated SEC of boiled lentil extract showed comparable elution patterns with clearly separated allergenic fractions. Pooling facilitates further chromatographic column fractionations in order to obtain highly purified lentil allergens.
**Discussion:** Although lentil consumption in the Western industrialized world is low compared with the (sub) tropical regions, the prevalence of lentil sensitization is likely to grow because of the increased consumption in these areas. Primary sensitization by other legumes may also contribute to unexpected lentil related symptoms. Blot results indicate that the same lentil allergens are recognized by IgE from patients from distinct geographical areas. This research describes another example of the development of an allergy against plant food allergens that are newly introduced in our “western”daily diet.

[1)] EuroPrevall, projectnumber 514000. EuroPrevall is a large multicentre study on the epidemiology of food allergy in Europe.

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**Sensitization to *Ascaris*, *Dermatophagoides pteronyssinus* and *Blomia tropicalis* in the tropical Island of Martinique.**

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**Background:** We have previously demonstrated that the presence detectable specific IgE to *Ascaris,* increases the prevalence of positive skin tests to common aeroallergens, especially mites and cockroaches, and to food allergens, such as shrimp in Martinique. **Objective:** The objective of this study was to analyse the correlation between sensitization to *Ascaris*, and *in vitro* sensitization to *Dermatophagoides pteronyssinus*, and *Blomia tropicalis* in a population of patients residing in the tropical Island of Martinique. **Material & Methods:** 607 consecutive patients (321 females and 286 males) were evaluated at a local allergy clinic for allergic respiratory complaints from February 2003 to March 2007. Mean age was 20.36 (1-75 years). Specific IgE was determined by the CAP method (Phadia). **Results:** Patients were divided into 2 groups: Group 1: 333 patients with < 15 years of age, and Group 2: 274 patients with > 15 years of age. Mean total IgE levels in the studied population was 752.03 kU/L (2-39.888); 238 patients (39.2%) had a positive specific IgE determination to *Ascaris*; 387 (63.76%) to *D. pteronyssinus*; 399 (65.73%) to *B. tropicalis* and 218 to *B. germanica* (35.9%). In Group 1: mean total IgE level was 987.83 (2.13-39.888); 147 (44.14%) were positive to *Ascaris*; 234 (70.27%) to *D. pteronyssinus* and *B. tropicalis* and 134 (40.24%) to *B. germanica*. In group 2, mean total IgE: 465.46 kU/L (2-8.453); 91 (33.2%) were positive to *Ascaris*; 153 (55.84%) to *D. pteronyssinus*; 165 (60.22%) to *B. tropicalis* and 84 (30.66%) to *B. germanica*. In group 1, 146 of the 147 *Ascaris* positive patients (99.32%) were positive to at least 1 mite and 142 to both species; 186 patients in this group were negative to *Ascaris*; in this group 98 (52.69%) were positive to mites ($p<0.001$). In group 2: 91 (33.21%) were positive to *Ascaris* and among them, 81 were positive to at least 1 mite species (89.01%); in the *Ascaris* negative group (183), 97 (53%) were positive to at least one mite species ($p<0.001$). **Conclusions:** Sensitization to *Ascaris* seems to be a significant risk factor for sensitization to mite allergens in the tropics.

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**Allergen specific IgG antibody levels modify the relationship between allergen specific IgE and current asthma**

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**Background:** Our previous studies have revealed that increasing levels of IgE antibodies to inhalant allergens, like cat in symptomatic patients, are associated with an increased likelihood for asthma. However, the role of allergen specific IgG and $IgG_4$, in relation to IgE antibodies, has yet to be determined.
**Aim:** To investigate the association between allergen specific IgE, IgG and $IgG_4$ antibodies with current wheeze in two geographically different population-based birth cohorts.

**Methods:** Four hundred and seventy three children in Manchester, England in a population-based birth cohort (MAAS), and 1336 children in Perth Western Australia in a population-based birth cohort (RAINE), were followed from birth to age 5 years (England) and age 13 years (Australia). Validated questionnaires were administered to collect information on parentally-reported wheezing. Current wheeze was defined as wheezing in the previous 12 months. Cat specific serum IgE, IgG and $IgG_4$ antibodies and Fel d 1 specific IgG and $IgG_4$ antibodies were measured using ImmunoCAP® (Phadia AB, Sweden) contemporaneous with clinical outcomes.
**Results:** The predicted risk for current wheeze increased 1.57-fold (95% CI 1.27 – 1.95), per logarithmic increase in cat specific IgE level, in England, and 1.29-fold (95% CI 1.19 – 1.39) in Australia. Fel d1 specific IgG had no significant effect on current wheeze. In a multivariate analysis, cat specific IgE increased the risk, and Fel d 1 specific IgG decreased the risk for current wheezing in both populations (table 1). There was no obvious effect by IgG4 antibodies on these symptoms.

**Table 1.** Predicted risk for current wheeze using cat specific IgE and Fel d 1 specific IgG antibody levels.

| | Cat specific IgE | | Fel d 1 specific IgG | |
|---|---|---|---|---|
| | OR | 95% CI | OR | 95% CI |
| England | 1.78 | 1.39 - 2.26 | 0.40 | 0.19 - 0.84 |
| Australia | 1.33 | 1.23 - 1.44 | 0.78 | 0.65 - 0.95 |

**Conclusion:** While cat specific IgE antibody levels significantly increased the risk for current wheeze in both cohorts, Fel d 1 specific IgG antibodies significantly decreased this risk, whereas IgG4 antibodies to cat had no effect on symptoms. IgG antibodies were raised in many individuals irrespective of symptoms.

## 98
### Diagnosing hypersensitivity reactions to cephalosporins in children

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**Background:** Cephalosporins may cause hypersensitivity reactions, such as maculopapular, angioedematous and urticarial ones, classifiable as immediate or nonimmediate according to the time interval between the last drug administration and the onset. Immediate reactions occur within one hour; nonimmediate ones occur after more than one hour.
**Objective:** To evaluate the usefulness of immediate- and delayed-reading skin tests, patch tests, serum specific IgE assays, and challenges in diagnosing hypersensitivity reactions to cephalosporins and to clarify the pathogenic mechanism of such reactions.
**Methods:** Children with immediate manifestations underwent immediate-reading skin tests with penicillin reagents (penicilloyl-polylysine, minor determinant mixture, benzyl-penicillin, amoxicillin, and ampicillin) and any suspect cephalosporins, serum specific IgE assays, and, in case of negative results, challenges; some children with negative results underwent challenges and re-evaluations.
Children with nonimmediate manifestations were assessed by both delayed-reading skin tests and patch tests, and, in case of negative responses, by provocation tests.
**Results:** We evaluated 148 children with hypersensitivity reactions to cephalosporins, mainly cefaclor and ceftriaxone; 105 had suffered nonimmediate manifestations (mostly urticarial eruptions and maculopapular rashes), and 43 immediate ones (anaphylactic shock, urticaria and/or angioedema, and erythema). None of the nonimmediate reactors presented patch test and/or delayed skin test positivity; only one subject displayed immediate positive responses to penicillin skin test reagents. Among the 104 negative patients, 96 underwent challenges: 95 children tolerated them and one reacted to the cefaclor suspension and tolerated the challenge with a cefaclor capsule.
In the first allergologic work-up, 33 of the 43 children with immediate reactions displayed skin-test positivity. Of the 10 negative patients, 7 underwent challenges, followed by therapeutic courses and re-evaluations in 4. All challenges and therapeutic courses were tolerated; in the re-evaluation, one girl presented positive skin tests to both the responsible cephalosporin and penicillin reagents. Overall, an IgE-mediated hypersensitivity was diagnosed in 34 (79%) of 43 subjects.
**Conclusions:** Extremely few nonimmediate manifestations associated with cephalosporin therapy are actually hypersensitivity reactions, while most immediate reactions to cephalosporins are IgE-mediated. Cephalosporin skin testing is a useful tool for evaluating such reactions.

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### The New GA²LEN Pan-European standard prick test: First results

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GA²LEN (Global Allergy and Asthma European Network) is an alliance of allergy centres throughout Europe, aiming to improve research, clinical care and reduce the burden of allergy. Unlike until today in contact allergy no European standard existed for inhalant allergies. This is based on the opinion that only locally relevant allergens should be tested. However, mobility is continuously increasing in Europe. It was, therefore, imperative, to develop a testing panel to be used as a standard in European allergy centres covering the most important inhalant allergens within Europe.

The pan-European prick test project (PEP) was thus designed as a multicenter including 16 centres in 13 countries of all regions in Europe. Inhalant allergens thus included all those frequent in all regions: Aspergillus, Cat, Dog, Dermatophagoides pteronissinus, Dermatophagoides farinae, Blatella, Hazel, Alder, Birch, Plane, Cypress, Olive, Grass mix, Artemisia, Ambrosia, Alternaria, Cladosporium, Parietaria represent the standard PAN European prick test as core panel. Histamine 1:100 was used as a positive control, diluent as a negative control.

A test was regarded positive if the value calculated was ≥ 3 mm or 50 % of the control.

A total of 2026 valid and complete sets were used for statistical analysis. In summary, the results of this study demonstrate that sensitizations to allergens regarded untypical to region frequently occur. Examples are Birch 9.17 % in Mediterranean countries or Blatella 10.35 % in Scandinavia or Ambrosia 15.10 % in the Netherlands. Furthermore, with exception of Plane in Scandinavia (1.39%) no sensitization was below 2% which is regarded as the cut off level for the inclusion in standard tests in contact allergy. In conclusion, the results strongly support the value of the use of the GA²LEN standard prick test. Thereby, unexpected sensitizations during short-term travel to foreign countries or previously not recognized sensitizations in immigrants will be detected most efficiently.

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## 100
### Characterization of monoclonal antibodies against Bet v 1 and their use as tools for quality assessment of recombinant Bet v 1 derivatives

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**Background:** Hypoallergenic recombinant Bet v 1 derivatives, such as a rBet v 1 folding variant (FV), are promising candidates for allergen specific immunotherapy. Phase II and III clinical studies have demonstrated the clinical efficacy of Bet v 1-FV. It is important to develop adequate test systems to ensure the consistent quality of different batches of such a preparation in respect to its immunological properties. Investigation of the binding features of monoclonal antibodies (mab) raised against natural Bet v 1 and rBet v 1-FV demonstrates their suitability for this purpose.
**Methods:** Mab were raised in BALB/c mice with nBet v 1 and rBet v 1-FV respectively. Their binding features were investigated with nBet v 1, rBet v 1-WT (wild type), rBet v 1-FV and two fragments (aa 1-73 and 74-159) of rBet v 1 by

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ELISA and Western blots of birch pollen extract. The epitopes were analyzed with biotinylated peptides of Bet v 1 and by inhibition-ELISA. Two-site binding assays were established with mab of different epitope specificities.
**Results:** 12 mab raised against nBet v 1 and 31 mab against rBet v 1-FV were included in the investigation. All nBet v 1-induced mab recognized rBet v 1-WT, but only 7 mab bound to the rBet v 1-FV to a similar degree. In contrast, almost all rBet v 1 FV-induced mab recognized nBet v 1 and rBet v 1-WT, only one binds exclusively to the rBet v 1-FV. The epitopes of the majority of mabs were identified with the peptides thus proving their sequential nature. Two-site-binding assays enable the quantification of all three Bet v 1-molecules or nBet v 1 and rBet v 1-WT on the one hand and rBet v 1-FV on the other. A panel of mab with different epitope specificities and binding characteristics revealed the consistency of different production batches of rBet v 1-FV.
**Conclusion:** A panel of mab raised against Bet v 1 and rBet v 1-FV provide valuable and precisely defined tools for the quantification and quality assessment control of the rBet v 1 derivative rBet v 1-FV. Furthermore the binding characteristics of mabs raised against rBet v 1-FV demonstrate the high potential of this derivative to induce antibodies against the properly folded molecule.

## 101
**The evolution of allergenicity in the prolamin and Bet v 1 superfamilies**

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**Background**: The massive increase of sequence data stimulated a classification of proteins into families and superfamilies. The distribution of allergens into these families is highly biased. They are found in only 2% of the 9318 families defined by the Pfam protein family database (http://pfam.sanger.ac.uk/).
**Experimental methods**: We studied allergens of the prolamin and the Bet v 1 superfamilies in their evolutionary context to elucidate the emergence of allergenicity. Sequences of representative proteins of the families of each of the superfamilies were aligned using ClustalX 1.83. Sequence identity matrices and neighbor-joining phylogenetic trees were generated from these alignments and visualized using TreeView 1.6.6. Allergenicity and cross-reactivity data of family members was extracted from the literature.
**Specific findings**: Numerically, the prolamin superfamily is the most prominent of all protein families that contain allergenic members while the Bet v 1 superfamily ranks 7th. Allergens were found in 4/8 prolamin families: 2S albumins, non-specific lipid transfer proteins (nsLTPs), cereal prolamins and alpha-amylase/trypsin inhibitors. These 4 families have little sequence identity and hence do not cross-react. 2S albumins share only 7-25% of their sequences with nsLTPs. Cross-reactivities within the 2S albumins or the nsLTPs are generally low. In contrast, allergens of the Bet v 1 superfamily are restricted to the PR-10 family, one of its five member families. Sequence and structural similarities and hence cross-reactivities are high between Bet v 1 and Bet v 1 homologues.
**Conclusions**: Allergens can be found in 50% of the prolamin families indicating a tendency of a protoprolamin to be allergenic. Allergenicity seems to be an intrinsic molecular property that was already present in such an ancestral molecule. In the Bet v 1 superfamily allergenicity appears to have emerged only in the PR-10 family. Studies of allergenic and non-allergenic members of protein families should eventually lead to a new understanding of allergenicity.

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## 102
**Interaction of the major grass pollen allergen Phl p 1 with the respiratory interphase: Activation, uptake and transport**

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The respiratory epithelium forms the first line of contact and interaction between airborne particles, e.g. grass pollen, their released proteins and the innate immune system. We studied the immunological mechanisms involved in the interaction of respiratory epithelial cells and Phl p 1 as a model for an important grass pollen allergen.
Natural Phl p 1 was able to activate the epithelial cells for IL-8 release in a protease-independent manner under physiological conditions. The allergen itself can be cleaved into fragments by mucosal secretions such as BAL fluid, nasal secretion and supernatants from neutrophils and mast cells on the epithelial surface. In oder to study the allergen uptake and transport through the epithelial barrier, we used the alveolar cell line A549 and the bronchial cell line Calu-3 and performed FACS analysis and confocal microscopy. After allergen uptake, the A549 cells showed no colocalisation of Phl p 1-containing particles and lysosomes and the allergen was then released into the culture medium in a time-dependent manner. This suggests a transcytotic mechanism. In contrast to the A549 cells, the Calu-3 cells revealed colocalisation of Phl p 1-containing vesicles and lysosomes indicating that the allergen might be processed. This is in line with the identification of HLA-DR and CD86 molecules on Calu-3 cells, serving for antigen presentation and as costimulatory signal, respectively. Both molecules are lacking on A549 cells. Investigations of human lung biopsies with Phl p 1 showed a small allergen uptake by the epithelial cells of the alveolar region, while in the bronchial epithelium an allergen uptake was clearly detectable. Further studies are in progress to follow the allergen processing of the allergen on the molecular level.
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## 103
**Has Ambrosia senzitization any triggering effect on allergies?**

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The overall spreading of Ambrosia pollen in Europe (recently even in Southern Scandinavia) is an important indicator of increasing environmental pollution and global warming. It is not its own allergenicity and sensibilizing effect only but the suggested triggering effect on further senzitizations , too, what concerns allergologists and patients alike. Firm proofs of this assumption are however still lacking.
We have compared sensitization profiles and prevalence of allergies in 172 patients ( 150 adults and 22 children) living either in heavily (SE and SW) or in less (N) Ambrosia polluted areas of Hungary. Patients have been randomly selected for the study at the outpatient allergy clinics.
Methods used were : standardized questionnaires + in vitro specific IgE determinations (inhalative and food allergen panels, 49 items)
Most important conclusions were: in heavily polluted areas Ambrosia senzitization of the total population was 55-65 % , (higher in children than in adults), while in more preserved areas 15-20 % only (in these areas grass and Parietaria senzitizations were highest). Average for the whole population was 35%. No unsensitized (against any allergen in the panel) children have been found, while in adults the ratio was close to 10%.
The highest prevalence of respiratory allergic symptomes was observed in the Eger (N) area where Ambrosia sensitization was lowest, making the decisive role of the pollen unlikely. Polysensitization (2 or more specific IgE , class 2 or higher in the inhalant panel ) was characteristic for half of the adult population but not linked to Ambrosia pollen concentrations.
In spite of the clinical experience of several allergologists in Hungary , these preliminary data (subject to further evaluation) suggest that there is no obvious and direct relationship between Ambrosia spreading and non-Ambrosia mediated allergies . This , however does not make eradication projects going on in Central-East Europe senseless , as Ambrosia sp. still remains the major outdoor allergen is those areas.

## 104
**Genomics of *Aspergillus fumigatus* allergens**

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*Aspergillus fumigatus* (*A. fumigatus*), a filamentous saprophytic mould ubiquitously present in our environment, is an opportunistic human pathogen associated with a vast range of allergic diseases including allergic asthma and allergic bronchopulmonary aspergillosis (ABPA). The allergen repertoire of *A. fumigatus* obtained by cDNA cloning is still the most complex repertoire described so far and includes more than 80 putative IgE-binding proteins. However, as expected from cDNA cloning, many of the allergen sequences reported does not represent full-length clones. The recent publication of the complete *A. fumigatus* genome sequence[1] provides an excellent tool to validate the published allergen

sequences, to predict the full length sequence of truncated cDNAs, and to assess the occurrence of allergen orthologues across the fungal kingdom.
A detailed comparison of the recently cloned *A. fumigatus* thioredoxins (Asp f 28, Asp f 29), cyclophilins (Asp f 11, Asp f 27), and Phi A cell wall protein (Asp f 34) sequences with the genome sequence showed an almost 100% identity. The corresponding proteins have been recombinantly produced and evaluated and for their IgE-binding capacity *in vitro* and for their ability to elicit positive skin tests *in vivo*. This is not the case for other postulated *A. fumigatus* allergens like Asp f 16. Although the Asp f 16 sequence is largely identical to the Asp f 9 sequence in extended regions, the predicted Asp f 16 polypeptide does not appear to be encoded by the *A. fumigatus* genome. From the genomic analysis it appears that the published Asp f 16 protein sequence results from frame shift or other sequencing errors. The postulated Asp f 56 kDa allergen derived from a short peptide sequence is not predicted to be encoded in any of the *Aspergillus* genomes sequenced, and Asp f 15 results to be identical to Asp f 13 because no other gene matching this sequence exists in the genome. Moreover, because at present more than 40 fungal genomes are fully sequenced, comparative genomics allows surveying the occurrence of allergen orthologues across the fungal kingdom as predictors for cross-reactivity. These few examples highlight the relevance of genomics to everyday research.

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[1] Nierman WC, Pain A, Anderson MJ, Wortman JR, Kim HS, Arroyo J et al. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. *Nature* 2005, 438: 1151-1156.

## 105

### Doxycycline reduces MMP9, ECP, MPO and nasal polyp size, in a double-blind, randomized, placebo controlled, multicenter trial

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**Background:** Recent evidence suggests that a chronic microbial trigger might play an important role in the pathogenesis of chronic rhinosinusitis with nasal polyposis (NP). For treatment, doxycycline was selected based on its antimicrobial effect and anti-inflammatory effect. The aim of the study is to evaluate the effect of oral doxycycline on nasal polyp size, nasal peak inspiratory flow (nPIF) and on the local inflammation in nasal polyps.
**Methods:** In a randomized, double-blind, placebo controlled, parallel group, multicenter study, 32 subjects with bilateral nasal polyps were randomized to receive placebo or antibiotics at a dose of 200 mg doxycycline at day one, followed by 100 mg doxycycline from day two till day 20. The effect was assessed by endoscopic evaluation of polyp size, symptoms, peripheral eosinophil counts, local IgE, MPO, MMP-9 and ECP levels.
**Results:** Doxycycline treatment resulted in a significant decrease of the endoscopic nasal polyp score after 4 to 12 weeks after the start of the doxycycline treatment compared to placebo. The concentrations of IgE, MPO, MMP-9 and ECP decreased significantly in nasal secretions of doxycycline treated patients.
**Conclusion:** This is first study demonstrating the positive clinical effect of doxycycline on nasal polyposis, paralleled by a significant impact on markers of local inflammation and remodelling. Thus, in a clinical setting the use of doxycycline offers an additional advantage of providing both antimicrobial and anti-inflammatory effects with a decrease in nasal polyp size.

## 106

### The effects of cetirizine and its enantiomers on the transport of monoamines by human organic cation transporter 2 (hOCT2)

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Recently a pharmacokinetic interaction between cetirizine and pilsicainide was reported (Tsuruoka S. et al, CPET 2006 ; 79(4) :389-96) and explained partly as a consequence of competition for renal excretion mediated by organic cation transporter 2 (hOCT2). The concentration at which cetirizine was claimed to inhibit hOCT2 (0.1 µg/mL) was lower than the $C_{max}$ (*ca* 0.3 µg/mL) achieved following a therapeutic dose. In addition to its role in the renal excretion of xenobiotics, hOCT2 is also thought to play a role in the disposition of histamine. Histamine is inactivated by intracellular (cytosolic) histamine N-methyl-transferase and by diamine oxidase, stored in secretory vesicles at the plasma membrane. hOCT2 plays a key role in the transport of histamine from extracellular compartments into the intracellular space and its inhibition could lead to changes in the concentrations of extracellular histamine. The effects of cetirizine and its enantiomers on hOCT2 were further investigated.
The inhibition of hOCT2 by cetirizine and its enantiomers in S2 cells expressing hOCT2 was studied using creatinine as substrate. The $IC_{50}$s for cetirizine, levocetirizine and dextrocetirizine in this assay were 362 µM (167 µg/mL), 227 µM (105 µg/mL) and 499 µM (231 µg/mL), respectively. The inhibition of hOCT2 by cetirizine enantiomers was also studied in CHO cells expressing hOCT2 using tetraethylammonium as substrate**.** The Ki values obtained were 180 µM (83 µg/mL) and 680 µM (314 µg/mL), respectively, for levocetirizine and dextrocetirizine. The positive control inhibitor cimetidine gave the expected results confirming the validity of the test systems used.
The inhibitory concentrations in these studies represent free concentrations. Cetirizine is 89.2% and levocetirizine is 92.0 % bound to plasma proteins. The interaction of some drugs with transporters is dependent on the free drug concentration. It is therefore possible that less than 10% of the total plasma concentration of levocetirizine or cetirizine is available for interaction with transporters. The data show that cetirizine or its enantiomers only inhibit hOCT2 at concentrations 3-4 orders of magnitude greater than their free plasma concentrations. It is unlikely therefore that levocetirizine or cetirizine can affect renal drug elimination or interfere with histamine disposition through inhibition of hOCT2.

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### Targeting the extracellular membrane-proximal domain of membrane-bound IgE by passive immunization blocks IgE synthesis *in vivo*

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Allergic responses are due to the production of allergen-specific IgE bound to the high-affinity receptor for IgE on mast cells and basophils. Treatment of allergy with humanized anti-IgE antibodies leads primarily to a decrease of serum IgE levels. As a consequence, the number of high-affinity IgE receptors on mast cells and basophils decreases, leading to a lower excitability of the effector cells. The biological mechanism behind anti-IgE therapy remains partly speculative; however, it is likely that these antibodies also interact with mIgE on B cells and possibly interfere with IgE production.
In the present work we raised a mouse monoclonal antibody directed exclusively against the extracellular membrane-proximal domain of mIgE. The interaction between the monoclonal anti-mIgE antibody and the mIgE antigen receptor induces receptor-mediated apoptosis *in vitro*. Passive immunization experiments lead to a dramatic decrease of allergen-specific serum IgE during parallel application of recombinant Bet v1a, the major birch pollen allergen. The dramatic decrease of allergen-specific serum IgE might be related to tolerance-inducing mechanisms stopping mIgE-displaying B cells in their proliferation and differentiation. Based on these results, exclusively targeting human mIgE would be a reasonable approach to prevent allergic diseases with the advantage of inhibiting IgE secretion before production of secreted IgE starts.

## 108

### A specific mixture of short chain galacto-oligosaccharides and long chain fructo-oligosaccharides induces an anti-allergic immunoglobulin profile in infants at risk for allergy

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**Introduction:** In a prospective study in infants with a family history of atopy a specific prebiotic oligosaccharide mixture (90% short chain galacto-oligosaccharides and 10% long chain fructo-oligosaccharides (GOS/FOS) (IMMUNOFORTIS) reduced the cumulative incidence of atopic dermatitis at six months of age [1]. In a subgroup of these infants (n=84) it was possible to obtain a blood sample at six months of age to analyse the potential effect of these dietary oligosaccharides on the immunoglobulin profile.
**Materials & Methods:** In this prospective double-blind randomised, placebo controlled, study the infants received a hypoallergenic formula with either 8g/l GOS/FOS or 8 g/l maltodextrine (placebo) for six months. At three months of age, children were vaccinated against diphtheria, tetanus and polio (DTP). At six months of age total plasma levels of IgE, IgG1, IgG2, IgG3, and IgG4 as well as cow's milk protein (CMP) and DTP specific immunoglobulins were measured by ELISA.
**Results:** Supplementation of GOS/FOS has led to a significant reduction in plasma level of total IgE (p=0.007), IgG2 (p=0.029) and IgG3 (p=0.0343) immunoglobulins whereas no significant effect on IgG4 was observed. The plasma levels of CMP specific IgG1 were significantly decreased (p=0.015) in the GOS/FOS group. The levels of CMP specific IgE were very low and no effect of GOS/FOS supplementation could be observed. CMP specific IgG4 was not detectable in the samples. No influence of GOS/FOS supplementation was found on any vaccine specific antibody isotype levels. Follow up data obtained at 24 months of age indicated significant lower incidence of atopic dermatitis and infections in these children [2].
**Conclusion:** Evidently GOS/FOS supplementation induced an anti-allergic immunoglobulin profile in infants at high risk for allergic diseases, while desired specific immune responses were not affected. This indicates a potential role of oral GOS/FOS exposure for primary prevention of allergies.

1. Moro, G., Arslanoglu, S., Stahl, B., Jelinek, J., Wahn, U., and Boehm, G. (2006). A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 91, 814-819.
2. ESPGHAN 2007. Abstract follow up data.

## 109

### Combination vaccines for rhinovirus infections and allergy

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Allergens and rhinovirus infections are among the most common elicitors of asthma, a severe disabling disease affecting more than 300 million people worldwide. We report the construction of a recombinant combination vaccine for allergen- and rhinovirus-induced asthma based on rhinovirus-derived VP1, the surface protein which is critically involved in infection of respiratory cells, and a non-allergenic peptide of the major grass pollen allergen, Phl p 1. The VP1 hybrid molecule showed no IgE reactivity, did not induce basophil activation in allergic patients and hence lacked allergenic activity. Immunization of mice with the hybrid molecule did not sensitize against Phl p 1 but induced a VP1 and Phl p 1-specific Th1-like immune response accompanied by the production of VP1 and Phl p 1-specific IgG antibodies which protected against grass pollen allergy and rhinovirus infections. Using VP1 as a carrier protein, combination vaccines against the most common allergens and rhinovirus infections can be engineered.

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### Patterns of IgE, IgG, skin tests responses to Bet v 1 and birch pollen extract after immunotherapy with recombinant Bet v 1

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**Background:** We demonstrated that rBet v 1, nBet v 1 and birch pollen immunotherapy (IT) resulted in a significant reduction of symptoms compared to a placebo group. In the same patients (rBet v 1 (n=32), nBet v 1 (n=29), birch pollen (n=29), placebo (n=35))we evaluated changes in skin reactivity, specific IgE, IgG 1-4, IgM and IgA levels.
**Methods:** Skin prick tests (birch pollen :100 IR/ml, rBet v 1 and nBet v 1:50 µg/ml) were performed in quadruplicates at 4 time points : at enrolment, before IT and after one and two years of treatment. Serum samples were taken at these timepoints. Birch pollen and Bet v 1 specific IgE and IgG levels were quantified by immuno CAP. Birch pollen, r Bet v 1 and r Bet v 2 specific IgG 1-4, IgA and IgM were measured by ELISA.
**Results:** The changes from baseline in wheal diameters at one year after treatment for the 3 allergens, were significantly decreased in treated patient compared to patients under placebo ($p < 0.001$). r Bet v 1 IT induced a significantly greater decrease of skin reaction to birch pollen than birch pollen IT($p< 0.037$).
There was a marked increase of birch and Bet v 1 specific IgG 1 and IgG 4 for each active treatment group. Bet v 1 specific IgG confirmed these results. Bet v 1 specific IgG 2 increased after treatment but there was no relevant increases of Bet v 1 specific IgG 3, IgM or IgA levels. Calculated in an exploratory way, in the rBet v 1 group, the rise in IgG levels from the beginning of IT to one year later was correlated with the decrease of prick tests to rBet v 1 ($p = 0.018$) nBet v 1 ($p = 0.009$) and birch ($p = 0.003$). There was a positive correlation between the changes of total symptom score at the peak pollen season and the increase of IgG 1 ($p = 0.010$).
Three patients in the birch IT group developed Bet v 2 specific antibodies
**Conclusions:** Our results demonstrate that vaccination with recombinant allergen decreases skin reactivity to the naturel birch pollen allergen.

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### Phleum pratense alone is representative of Pooideae grass species for allergen specific immunotherapy

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**Background:** The Pooideae grasses constitute a large taxonomical subfamily with thousands of species. Due to structural and immunochemical similarity between homologous allergens grass pollen counts are communicated together. Immunotherapy for grass pollen allergy is often performed with mixtures of grass species; however, high clinical efficacy of grass pollen immunotherapy (SIT) using *Phleum pratense* alone has been demonstrated in several studies.
**Objective:** Study the immunochemical similarity of pollen extracts from different Pooideae grass species.
**Methods:** MagicLite solid phase immunoassay was used to measure a large number (n>13,000) of grass pollen allergic patients' serum IgE to eight Pooideae grass pollen extracts. IgE to eight Pooideae grass pollen extracts was measured by ADVIA Centaur solid phase immunoassay with or without inhibition by 2 mg *Phleum pratense* pollen extract. $IgG_4$ induced by SIT with *Phleum pratense* was measured to extracts of ten individual Pooideae grass species. Standard T cell stimulation assays using T cell lines from grass pollen allergic donors were applied to assess T cell cross-reactivity.
**Results:** A high correlation (0.86-0.98 Spearman rank correlation coefficient) was observed when comparing levels of IgE to *Phleum pratense* with those of pollen

extracts from individual grass species. *Phleum pratense* pollen extract inhibited IgE to other grass species more than 95% in most patients. SIT with *Phleum pratense* induced $IgG_4$ exhibiting a statistical significant correlation (0.92-0.99, Spearman) in the reaction with ten individual Pooideae grass pollen extracts. T cell lines specific for Phl p 1 and Phl p 5 both showed similar stimulation indices when stimulated with different grass pollen extracts indicating extensive T cell cross-reactivity between individual grass species.
**Conclusion:** The specificities of allergic patients IgE as well as $IgG_4$ induced by SIT are largely covered by Phleum pratense alone. T cell lines specific for the group 1 and 5 major allergens showed extensive cross-reactivity between grass species. One specie alone, e.g. *Phleum pratense*, therefore seems sufficient for specific immunotherapy against allergy to Pooideae grass pollens.

## 112

**Assessment of the clinical relevance of plant-glycan specific IgE by in vivo challenge with transgenic human lactoferrin expressed in rice.**

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**Background:** Poor biological activity of IgE antibodies directed to plant glycans (CCD) is well established. It has been suggested that low avidity of these IgE antibodies is the explanation. Their clinical relevance is still a matter of debate with potential impact on safe application of plant-derived pharmaceutical glycoproteins substituted with plant glycans. *In vivo* challenges are the only way to establish clinical relevance.
**Aim of the study:** We sought to evaluate the clinical relevance of IgE antibodies against plant glycans using human lactoferrin produced in rice (rHLF) in double-blind placebo-controlled oral challenges (DBPCOC).
**Methods:** Allergic patients with IgE antibodies against plant glycans were analyzed for the presence of IgE against rHLF. Relative avidity of IgE antibodies against plant glycans and against a major non-glycosylated grass pollen allergen rPhl p 5 was assessed by IgE inhibition assays. The potency of IgE to induce mediator release was assessed by basophil histamine release (BHR) and skin prick tests (SPT). Clinical relevance was evaluated by DBPCOC.
**Results:** Sera with IgE antibodies against plant glycans demonstrated IgE binding to rHLF but not to native lactoferrin. In a majority of the cases, rHLF induced histamine release. Compared to rPhl p 5, rHLF concentrations needed for biological activity of IgE were 5-6 orders of magnitude higher. Difference in avidity is unlikely to be the explanation for this observation, because very similar concentrations of allergen were needed to achieve 50% inhibition of IgE binding to both allergens. SPT and DBPCOC were negative in patients with potential clinical reactivity that volunteered to undergo these *in vivo* challenges.
**Conclusions:** Poor or no biological activity and lack of clinical relevance of IgE-binding plant glycans was demonstrated using human lactoferrin expressed in rice as a model. Low avidity of IgE antibodies is unlikely to be the explanation of this phenomenon. Pharmaceutical glycoproteins produced in plants are not a risk factor for their application in pollen allergic patients with plant glycan-specific IgE antibodies.

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**Role of IgE affinity and clonality for basophil activation and facilitated antigen presentation**

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**Background**: Type I allergy pathogenesis is tightly linked to pro-inflammatory processes that are triggered or sustained by complex-formation between allergen-specific IgE-antibodies, allergens, and cell-surface bound IgE-receptors, e.g. FcεRI for mast cell- and basophil-activation, and CD23 for facilitated antigen-uptake and -presentation (FAP). In sensitized individuals a broad variability of basophil activation exists, indicating substantial patient-to-patient variations either in the IgE-antibody repertoire and/or in the cellular response downstream of IgE-receptor ligation. To what extent individual IgEs in serum contribute to complex formation remains to be established. Using monoclonal recombinant IgE-antibodies we examined the effects of IgE-clonality and individual antibody affinities on complex formation in basophil activation tests (BAT) and FAP-mediated T-cell activation.
**Methods**: A panel of humanized Derp2-specific recombinant IgE-antibodies was cloned, characterized, and engineered with regard to affinity. Twenty recombinant IgEs against three non-overlapping Derp2-epitopes and ranging in affinity between 0.036 and 291 nM were used in various combinations, as artificial patients' sera of defined composition, in BAT and FAP-assays.
**Results**: The effect of antibody affinities on complex-formation/cellular activation depended on the individual antibody combinations. In BAT, cellular activation in the presence of two non-overlapping high-affinity IgEs was obtained at 1000-fold lower allergen concentration than required for complex formation with two low-affinity IgEs. However, one high- and one low-affinity IgE were as efficient as two high-affinity IgEs. Cellular activation by complexes with three non-overlapping IgEs was up to 10-fold more efficient than with two non-overlapping IgEs, at the same total IgE-concentration. Intermediate results were obtained with other IgE-combinations. In FAP-assays similar correlations were seen.
**Conclusion**: We demonstrate very directly that in BAT and FAP-assays complex-formation/cellular activation depends on the avidity of IgE/allergen/receptor complexes which, in turn, is governed by a combination of IgE-clonality and individual antibody-affinities. The finding that cellular activation only requires that one antibody of the specific IgE-repertoire is of high affinity indicates an important role for low-affinity IgEs in allergy (notably in cross-allergy), and entails that diagnostic assays should address the composition of IgEs in a patient's serum rather than just antibody titers. The finding may also explain why substantial patient-to-patient variations are commonly seen in connection with hypoallergenic allergen-variants.

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**Hazelnut oleosin: Identification and characterization of a novel hazelnut allergen**

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**Background:** Patients clinically reactive to peanut and/or tree nuts can score negative in various diagnostic tests. Recently, a new oilbody-associated allergen (oleosin) was reported in peanuts. Two hazelnut oleosin isoforms were detected in a cDNA library screening.
**Objective:** To determine the role of hazelnut oleosin in (severe) hazelnut allergy and the prevalence of oleosin-specific IgE in (tree)nut/seed allergic patients.
**Methods:** His-tagged hazelnut oleosin isoforms were expressed in *E. coli* and purified by affinity chromatography. IgE binding was tested by immunoblotting. Hazelnut, peanut, walnut, cashew and sesame oleosin were (partially) purified from extracts. Purity was assessed by gel staining (and Mass-spectrometry (MS) for peanut and hazelnut). IgE binding was tested by RAST with serum from patients with hazelnut and/or peanut ingestion related history (n=185). Semi-purified hazelnut oleosin and enriched oilbody fractions were used to generate rabbit polyclonal antibodies.
**Results:** Semi-purified natural oleosin was poorly soluble and thus could so far not be further purified. Major bands of 27(H1), 24 (H2), 18(H3) and 14(H4) kDa were analysed by MS. H1 corresponded with an unknown protein with minor quantities of both oleosin isoforms, H2 with 11S globulin, H3 and H4 with both oleosin isoforms. Immunoblots showed major IgE binding to two bands around 20 kDa and two bands around 40 kDa, which may correspond with oleosin mono- and dimers. Interestingly, the monomeric band seemed to be absent in (defatted) commercially available hazelnut extracts, and present in non-defatted extracts. 118/185 patients with hazelnut and/or peanut ingestion related symptoms and 31/171 in-house sera (mainly from people with inhalation-allergies) had a positive RAST to the semi-purified hazelnut oleosin fraction (>0.3 IU/ml). Both recombinant hazelnut oleosin isoforms were capable to inhibit IgE-binding to this fraction on blot. The semi-purified hazelnut oleosin fraction was capable of inducing histamine release in basophils using allergic patient sera (n=16).
**Conclusion:** Hazelnut oleosin might play a role as an allergen with a high prevalence and properties to evoke systemic reactions. The association with oilbodies may protect oleosins from rapid proteolysis after ingestion. Due to extensive defatting of extracts used for *in vitro* diagnostics, oleosin-specific IgE can easily be missed.

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### Almond and walnut 60S ribosomal protein P2: A new class of IgE-binding food protein with fungal aeroallergen cross-reactivity

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**Background:** Allergies to tree nuts are a serious health issue.
**Objective:** Identification of almond and walnut allergens.
**Method:** An almond cDNA library was screened and IgE immunoreactive clones amplified and expressed. The walnut homologue of the almond clone was PCR amplified, cloned, and expressed. The resultant products were screened with sera from self-reported almond and/or walnut-allergic patients by immunoblot and ELISA and compared to Fus c 1, a fungal (*Fusarium culmorum*) aeroallergen homologue. A rabbit polyclonal antibody was raised against the recombinant almond protein.
**Results:** Patient sera identified IgE-reactive almond and walnut cDNA clones that encode ~11,450 Dalton 60S acidic ribosomal proteins P2 (60S RP), designated as rPru du 5 and rJug r 5, respectively, that share 91% sequence similarity. Of 24 sera from almond- and/or walnut-allergic patients with self-reported severe allergy, 8 (33%) had IgE reactive with the recombinant proteins; 7 with both proteins and one with only walnut rJug r 5. The positive patients' sera, but no others, also reacted with, and were inhibited by, rFus c 1, a fungal 60S RP aeroallergen. Native 60S RP was demonstrated in both almond and walnut crude extracts.
**Conclusions:** 60S RP represents a new class of potential food allergens in plant-derived foods. A third of tested patients had IgE that bound almond (rPru du 5) and/or walnut (rJug r 5) homologues in *in vitro* assays. The data indicate considerable cross-reactivity between almond, walnut, and fungal homologues.
**Clinical Implications:** Nut allergic subjects sensitized to 60S RP may be at risk of experiencing allergic reactions to molds or vice versa.

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### X-ray crystallography of a monoclonal antibody complex with cockroach allergen Bla g 2 and identification of putative IgE epitopes.

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**Rationale**: The German cockroach allergen, Bla g 2, an inactive aspartic protease, has a bilobal three-dimensional structure, resulting from gene duplication. The antigenic surface of Bla g 2 was analyzed by co-crystallization of the allergen with monoclonal antibody (mAb) 7C11. Amino acid residues involved in IgE antibody binding were studied by site-directed mutagenesis.
**Methods:** The structure of recombinant Bla g 2 in complex with Fab' fragments of mAb 7C11 was solved by molecular replacement and refined using the heavy and light chain amino acid sequences. IgE antibody binding to *Pichia pastoris* expressed mutants was performed by multiplex technology and beta-hexosaminidase release from mast cells sensitized with sera from cockroach allergic patients. Folding of the mutants was tested by ELISA and CD spectroscopy.
**Results:** Bla g 2 binds to 7C11 through four loops including residues 60-70 plus R83, E86 and K132. Strong cation-pi interactions exist between K65, R83 and K132, and tyrosines in 7C11. Single mutations of these three cationic residues, D68a and E86 to alanine affected mAb and/or IgE antibody binding. Mutant K132A had the strongest effect on IgE and monoclonal antibody binding, without affecting the binding of an anti-Bla g 2 polyclonal antibody. The later was affected by mutations D68aA and R83A, reflecting differences in folding versus wild type, in agreement with the CD spectra. Bla g 2 forms a dimer in the complex, unusual for the aspartic protease family, which is stabilized by a four-helical bundle comprised of two α-helices from each allergen monomer. A unique di-sulfide bridge between C51a and C113, connecting two helices within each Bla g 2 monomer, contributes to this novel dimerization mode. Mutation of these cysteines plus the residues N52, Q110 and I114, involved in hydrophobic interactions, resulted in a protein that did not dimerize and induced less beta-hexosaminidase release than the wild type, suggesting a role of dimerization on allergenicity.
**Conclusions:** Analysis of the crystal structure of a Bla g 2-Fab' mAb complex enables to study the relevance of dimerization on allergenicity, the identification of IgE antibody binding epitopes and a rational approach for the design of hypoallergenic molecules.

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### Common traits and individualized patterns in the human IgE repertoire

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The human B-cell repertoire arises from a fine tuned process of the rearrangement of V gene segments together with D and J segments with imprecise junctions of these gene segments. This diversity is further enhanced by somatic hypermutations in the V region and heavy and light chain pairing.
In atopic individuals, a substantial part of the immunoglobulin repertoire is represented by IgE, an immunoglobulin which usually is found only in low amounts.
In an attempt to analyze the B-cell repertoire in atopic individuals with special emphasis on IgE rearrangements we have established a spectratyping technique of the hypervariable CDR-3 region of the IgM-, IgG-, and IgE immunoglobulins together with a quantitative RT-PCR and have analyzed PBMCs in more than 20 highly atopic individuals displaying IgE serum levels far above 1000 IU/ml.
This approach revealed that atopic individuals show only slightly elevated numbers of IgE-positive B-cells or plasma cells in their PBMCs but produce up to 1000 times more mRNA encoding for IgE, Atopic individuals display an identical usage of variable chains for IgE as for IgM and IgG with VH3b, VH4, and VH3a being dominant. Although the IgE repertoire was found biased in every patient, only few common expansions were identified between different individuals.
In accordance, sequence analysis of more than 400 CDR-3 sequences revealed individual rearrangements without signs of public responses despite the highly similar sensitization pattern to common allergens in these patients. The obtained IgE sequenced showed somatic hypermutation in 25 % of rearrangements, a level which is also seen in IgG immunoglobulins. Interestingly, in two patients identical CDR-3 rearrangements were found within IgE- and IgG-sequences arguing for the selection of identical CDR-3 regions to a given antigen as hypothesized in the induction of IgG by specific immunotherapy.
In conclusion, the IgE repertoire in the PBMCs of highly atopic patients show the imprint of individual rather than public responses without obvious bias of any given variable chain region. The evolution of this repertoire is suited to follow patients under various treatment modalities.

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### Robust long-term tolerance in a murine model of type I allergy through transplantation of genetically modified hematopoietic stem cells

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**Introduction:** No prophylactic approach for the prevention of allergy is currently available for widespread clinical application**.** Several experimental approaches for tolerance induction in allergy have been reported but are characterized by limited robustness and relatively short-lived effects. Molecular chimerism (i.e. transplantation of autologous hematopoietic stem cells modified to express the disease-causing antigen(s)) induces tolerance in several models of organ transplantation and autoimmune disease, but this strategy has not been explored in allergy. We thus investigated whether tolerance can be induced in allergy through

transplantation of syngeneic bone marrow retrovirally transduced to express an allergen.
**Methods:** BALB/c bone marrow cells (BMC) were retrovirally transduced *in vitro* to express Phl p 5 in a membrane-anchored fashion (transduction efficiency: 35-55%). Myeloablated BALB/c mice received 2-4x$10^6$ transduced BMC iv and were repeatedly injected sc with recombinant (r) Phl p 5 and rBet v 1 afterwards (0.5µg/mouse plus aluminumhydroxide, wks 6, 9, 12 and 22). Phl p 5 expression in recipient WBC and BMC was determined by FACS. Serum levels of allergen-specific isotypes were measured by ELISAs.
**Results:** All mice (n=20) transplanted with Phl p 5-transduced BMC developed high levels of molecular chimerism among all tested leukocyte lineages which remained stable for the length of follow up (40 weeks) (e.g. 11mean% Phl p 5+ B cells and 22% T cells, 25 wks post-BMT). Serum levels of Phl p 5-specific IgE and IgG1 remained undetectable in all chimeras throughout follow-up, while high levels of Bet v 1-specific IgE and IgG1 were measured. RBL assays revealed the absence of Phl p 5-specific degranulation in chimeras, whereas in contrast Bet v 1-specific degranulation was preserved. In T-cell proliferation assays chimeras showed specific non-responsiveness to Phl p 5 (n=3; $p<0.01$ vs non-transduced mice). In skin testing mice having received Phl p 5-expressing BM showed no mast cell degranulation upon Phl p 5 intradermal challenge in contrast to Bet v 1.
**Conclusion:** These proof-of-principle experiments demonstrate for the first time that molecular chimerism is a strategy that has the unique features of inducing tolerance in allergy that is robust and permanent.

## 119

### Idiopathic Anaphylaxis in Mast Cell Clonal Disorders is Potentially Linked to D816V Kit-Induced Hyper-Activation of Key Signaling Events for Mast Cell Activation

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Select subpopulations of patients with mast cell clonal disorders experience unexplained episodes of anaphylaxis. In patients with mastocytosis, at least one in ten experience spontaneous episodes of unexplained anaphylaxis. Further, we recently reported that a subset of patients diagnosed with idiopathic anaphylaxis had evidence of one or more minor criteria for mastocytosis and that c-kit mutational analysis was positive for the D816V activating mutation in 3 of 3 patients in CD25(+) bone marrow cells where the analysis was performed. We thus extended these observations by investigating the potential link between these reactions and the D816V mutation in Kit. It is known that antigen-mediated mast cell activation is synergistically enhanced by Kit activation following stem cell factor (SCF)-induced ligation; and that this response is dependent upon activation of an amplification signaling pathway mediated by phosphoinositide 3-kinase and the tyrosine kinase Btk, and coordinated by the transmembrane adaptor molecule NTAL (LAB/LAT2). We hypothesized that the D816V mutation in Kit may render mast cells hyper-responsive to antigen by further enhancing this amplification pathway, thus, accounting for the cases of anaphylaxis observed in mastocytosis patients. To investigate this possibility, a series of chimeric and full length Kit constructs were generated encoding both the full length and D816V forms of Kit, and these were used in transfection studies in 293T cells and human mast cells. In 293T cells, we observed that the D816V mutation resulted in a marked enhancement of the Kit auto-tyrosine phosphorylation compared to the wild type response and this was associated with a dramatic enhancement of NTAL phosphorylation. Furthermore, D816V Kit induced global phosphorylation of the tyrosines on Kit whereas wild type Kit only phosphorylated 2 of the 10 tyrosines. This hyper-phosphorylation of a critical intermediary of the integrated Kit/FcεRI signaling cascade would significantly enhance mast cell activation; and thus may provide insight into anaphylaxis associated with mast cell clonal disorders where the D816V mutation is present.

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### Disrupted Tight Junctions and Epithelial Susceptibility in Asthma

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Almost 20 years ago we suggested that chronic epithelial damage and aberrant repair were important factors in the pathogenesis of chronic asthma leading to a chronic wound scenario and subsequently suggested that this was linked to activation of the epithelial mesenchymal trophic unit (EMTU). We now provide evidence to show that epithelial tight junctions (TJs) are defective in asthma leading to a "leaky" barrier and that repair of this offers a novel approach for treatment. Identification of TJ proteins occludin and ZO-1 was assessed in bronchial biopsies using immunochemistry (IHC). In biopsies from normal subjects TJ proteins, were localised close to the apical surface of the epithelium forming consolidated areas of staining between individual cells consistent with formation of TJs. In contrast, in biopsies from mild, moderate and severe asthmatic subjects, irrespective of treatment, displayed patchy and irregular TJ staining with areas of epithelium lacking intercellular staining and in some instances the localization of the staining was completely altered with cytoplasmic and perinuclear staining. Since there was no difference in mRNA for ZO-1 or occluding between the normal and asthmatic biopsies and Western blots of whole cell lysates indicated that asthmatic cultures had significantly reduced levels of ZO-1 protein, and a trend for lower levels of occludin, we conclude that the TJ disruption is post-translational. Epithelial cells brushed from the airways and differentiated at an air liquid interface for up to 28 days also showed aberrant TJ formation by IHC and was associated with reduced transepithelial resistance (TER) suggesting that the barrier defect in the asthma-derived cultures was unlikely to be a simple consequence of a slower process of differentiation. There was also a significant relationship between the airway reactivity ($PC_{20}$ methacholine) *in vivo* and impaired barrier function *in vitro* ($R^2$= 0.693, $p<0.01$). EGF is a pleiotropic cytokine that is well known for its ability to promote cell proliferation but has also been shown to improve epithelial barrier function using in vitro and in vivo models of oxidant injury. In contrast to normal epithelial cultures, EGF caused a significant improvement in TER when applied to the apical surface of asthmatic cultures without affecting proliferation or goblet cell number and was accompanied by a marked increase in TJ protein staining. When L47A EGF, an analogue free from fibroblast proliferative activity, was applied to asthma-derived cultures it also restored TJ integrity and improved barrier function. Almost identical results were obtained with KGF which has no effect on fibroblasts. Finally, asthmatic when compared to normal cultures exposed to tobacco smoke extract exhibited greater increases in TER that could be prevented or reversed by the three growth factors. We conclude that asthmatic bronchial epithelium exhibits impaired TJ formation that could underlie the end organ expression of asthma, and that this impaired barrier function can be reversed by epithelial-directed growth factors suggesting a new approach to asthma treatment.

## 121

### Expression of calcitonin gene-related peptide in allergic tissue reactions

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The key mediators regulating the vascular changes characteristic of human late-phase allergic reactions have yet to be defined. We recently showed that the potent vasodilator, calcitonin gene-related peptide (CGRP), as well the CCR4 agonist CCL17 (TARC), were highly expressed in the airway wall (including the epithelium and submucosa) in human late asthmatic reactions induced by allergen-derived T cell peptides. Here we provide evidence that CGRP is produced by TARC-stimulated airway epithelium as well as inflammatory cells, in vivo and in vitro, and that this neuropeptide is also expressed in cells infiltrating whole allergen-induced cutaneous late phase allergic reactions. When BEAS-2B and A549 epithelial cell lines were stimulated with CCL17 there was a time- and dose-dependent increase (up to $10^5$ fold) in both alpha and beta CGRP mRNA (by RT-PCR) and the protein product (by quantative immunoflouresence and ELISA). Similar results were obtained after stimulation with IL-13, IL-1-beta and TNF-alpha. Immunoflourescent studies using both BEAS-2B cells and airway epithelial cells from biopsies from asthmatics indicated that TARC-induced epithelial cell CGRP production is via a CCR4-dependent mechanism. In atopic skin challenged with whole allergen the peak of the late phase reaction (6 hours) was associated

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with the maximal infiltration of CGRP+ cells which declined in number as the oedema and swelling resolved. Double immunoflouresence indicated that CGRP+ inflammatory cells in skin late reactions were predominantly neutrophils and CD3+ lymphocytes. In vitro, cytokine- and TARC-stimulated neutrophils could also be rendered CGRP+, but to a variable extent. Similar results in allergen-challenged skin were obtained with the potent vascular permeability factor, vascular endothelial growth factor (VEGF). Thus, VEGF+ cells infiltration was maximal at 6 hours but declined at 24 hours by which time the late reaction was significantly less pronounced. Taken together these data support the hypothesis that vascular dilation and leakage characteristic of allergen-induced late-phase allergic reaction are influenced by CGRP and may also involve VEGF.

## 122

### Anaphylaxis in patients with IgE ab to Galactose alpha-1-3 Galactose, can occur on exposure to mammalian proteins (including the monoclonal ab Cetuximab) which are glycosylated with this major xenoantigen

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Cetuximab is a chimeric mouse human mAB which is approved for the treatment of head and neck as well as colon cancer. In an area including Tennessee, N. Carolina, Arkansas and Virginia this mAB has been associated with severe hypersensitivity reactions in as many as 20% of patients. Using Phadia CAP coated with Streptavidin we developed an assay for IgE ab to cetuximab and found titers from 0.38-140 IU/ml. In pretreatment sera from 76 patients 18 were positive and 17 of these 18 had had a reaction to the first treatment severe enough to preclude further treatment ($p<0.001$).

Digestion of the cetuximab molecule established that the IgE ab was binding to the F(ab)2 fragment and further that the binding was abolished if the same amino acid sequence was expressed in CHO cells instead of the mouse myeloma cell line (SP2/0) used for the commercial product. The SP2/0 cell line can express the sugar Galactose alpha-1-3 Galactose (alpha-gal) but CHO does not. The fact that these IgE ab were binding to this xenoantigen was further confirmed by carrying out inhibition studies with soluble alpha-gal.

In keeping with the known species distribution of alpha-gal these IgE abs also bind to proteins from cat, dog, beef, pork and cows milk. Furthermore these antibodies can be depleted by >95% using a protein that is heavily glycosylated with alpha-gal (porcine thyroglobulin). We have now identified ten patients in Virginia, who have IgEab specific for alpha-Gal and who presented to clinic with a history of recurrent anaphylaxis or angioedema related to eating beef.

Although the reason why these IgE ab are common in this area of USA are not clear possible causes include histoplamosis, helminth infections and tick bites. The results establish that pre-existing IgE ab to alpha-Gal creates a risk of anaphylaxis on exposure to both natural and recombinant proteins that are glycosylated with this sugar.

## 123

### T-cells and eosinophils are crucial in the remodeling of bronchial smooth muscle cell layer in asthma

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**Background:** Pathological remodeling of bronchial smooth muscle cell (SMC) layer is a major feature of asthma. The structural changes refer to the size, mass and the number of SMC, with increased death as well as the proliferation index of myocytes. This process leads to thickening of the bronchial wall and contraction/relaxation disorder.

**Aim of the study:** Investigation of the mechanisms of bronchial SMC proliferation and death as well as the in vivo relevance of these processes in asthma.

**Methods:** Cultured human bronchial primary SMC were stimulated with various cytokines (IFN-γ, TNF-α, soluble Fas-Ligand (sFas-L), soluble TNF-related apoptosis-inducing ligand (sTRAIL), TGF-β, IL-13), histamine, eosinophil cationic protein (ECP), as well as activated Th1 and Th2 cells supernatants and eosinophil lysates. The effect of dexamethasone in the cell cultures was also observed. The viability was measured by ethidium bromide uptake. Proliferation was assessed by incorporation of [$^3$H] thymidine. Expression of death receptors (TNFR1, TNFR2, TRAIL1, TRAIL2, Fas, FasL), intracellular caspase-3 activation and the cell membrane inversion by annexin-V staining were investigated by flow cytometry. The morphological characteristics of apoptosis (nuclear condensation, DNA fragmentation) were assessed by immunocytology methods. Bronchial biopsy specimens from asthmatic subjects were stained and investigated for apoptotic features.

**Results:** IFN-γ, TNF-α, TRAIL and Fas-L significantly induced apoptosis, whereas ECP induced rapid necrosis of bronchial SMC. IFN-γ upregulated Fas and both TNF receptor 1 and 2, rendering the bronchial SMC susceptible to apoptosis. IFN-γ, TRAIL, sFas-L, as well as the activated T-cell supernatants significantly increased the intracellular caspase-3 activity and annexin-V positivity in the stimulated SMC. TGF-β significantly increased bronchial SMC proliferation, whereas IL-13 and histamine did not show any effect neither alone nor in combination with TGF-β. IFN-γ and dexamethasone potently inhibited the bronchial SMC proliferation. TGF-β efficiently restored suppression of bronchial SMC proliferation by dexamethasone, but not by IFN-γ. Increased expression of TRAIL in asthmatics, but not in non-asthmatic individuals was demonstrated in situ. The apoptosis receptors TRAILR1 and TRAILR2 were expressed in SMC and epithelial cells both in healthy and asthmatic biopsies. Apoptotic features of SMCs in vivo were prominent in fatal asthma.

**Conclusions:** Bronchial SMC represent an essential target of the inflammatory attack by T-cells and eosinophils. The bronchial tissue injury and induction of severe dysrelaxant pathology in the bronchi is determined by the balance between apoptosis and proliferation influenced by activated T-cells and eosinophils thus becoming a novel window for therapeutic interventions.

## 124

### A new twist in the pharmacology of non-steroidal anti-inflammatory drugs

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Non-steroidal anti-inflammatory drugs (NSAIDs) are analgesic and anti-inflammatory by virtue of inhibition of the cyclooxygenase (COX) reaction that initiates biosynthesis of prostaglandins. During a project defining the role of COX isoenzymes in allergic airway inflammation, it was observed that the profile of activity of diclofenac was distinctly different that that of the other tested NSAIDs. The observation gave rise to the hypothesis that diclofenac might be an antagonists of the thromboxane (TP) receptor.

Functional responses due to activation of the TP receptor were studied in isolated airway and vascular smooth muscle preparations from guinea pigs and rats as well as in human platelets. Receptor binding and signal transduction of the TP receptor was studied in HEK293 cells.

Diclofenac concentration-dependently and selectively inhibited the contraction responses to TP receptor agonists such as prostaglandin $D_2$ and U-46619 in the tested smooth muscle preparations, and also inhibited aggregation of human platelets induced by TP receptor activation but not by other stimuli. The competitive antagonism at the TP receptor was confirmed in the binding studies and at the level of signal transduction. Moreover, the selective COX-2 inhibitor lumiracoxib shared this activity profile, whereas a number of standard NSAIDs and other coxibs did not.

Diclofenac and lumiracoxib, in addition to being COX unselective and highly COX-2 selective inhibitors, respectively, displayed a previously unknown pharmacologic activity, namely TP receptor antagonism. Development of COX-2 selective inhibitors with dual activity as potent TP antagonists may lead to coxibs with improved cardiovascular safety, as the TP receptor mediates cardiovascular effects of thromboxane $A_2$ and isoprostanes.

## 125

**Autoallergy – a novel pathomechanism in chronic urticaria.**

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Patients with chronic urticaria (CU) are reported to frequently exhibit IgG autoantibodies against thyroid antigens such as thyroid peroxidase (TPO). We, therefore, speculated that some CU patients also express IgE autoantibodies against TPO and that CU in these patients may be autoallergic, i.e. due to skin mast cell activation by IgE-anti-TPO. To test this hypothesis we have developed an ELISA assay, which allows for the quantification of IgE-anti-TPO serum levels. We found that 18% of 305 CU patients screened (but none in the matched healthy control group) exhibit IgE-anti-TPO of more than 8 IU/ml. One third of these CU patients showed IgE-anti-TPO levels of >20 IU/ml, i.e. >2-fold more than the highest level found in healthy controls. Interestingly, thyroidectomy in two IgE-anti-TPO+ CU patients resulted in a continuous drop of IgG- and IgE-anti-TPO levels and complete remission of CU within 8 weeks after surgery, indicating that IgE-anti-TPO may be relevant for the induction of skin mast cell degranulation in CU. Furthermore, we found that preincubation of mast cells with IgE-anti-TPO and challenge with TPO results in strong mast cell degranulation and release of preformed mediators, supporting the view that IgE-anti-TPO-mediated mast cell activation may be a functionally relevant pathomechanism in CU. In summary, our findings suggest that CU in some patients may be autoallergic, i.e. due to IgE against self.

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**Th1 / T Reg Adjuvants For Sublingual Allergy Vaccines**

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**Rationale:** There is a major interest in identifying adjuvants for sublingual allergy vaccines with the aim to enhance allergen-specific tolerance.
**Methods:** Candidate adjuvants were screened using cocultures of human or murine dendritic cells (DC) with naïve $CD4^+$ T lymphocytes. Patterns of cytokine production and DC maturation were analysed by cytofluorometry. Quantitative PCR analysis of Fox p3/GATA3/TBet/IL4/IFNγ/IL10/TGFß genes was performed to assess Th1/Th2/T Reg lymphocyte polarization within 7 days. Selected adjuvants were tested *in vivo*, in a therapeutic murine model of sublingual immunotherapy (SLIT) in BALB/c mice sensitized with ovalbumin. Whole body plethysmography, lung histology and a detailed analysis of immune responses in blood, spleen and cervical lymph nodes were used as readouts
**Results:** Fifty ligands for major Toll like receptors (TLR2, 3, 4, 5, 7/8), as well as biological candidate adjuvants (ie over 15 strains of probiotics) have been tested. Both *Lactobacillus plantarum*, 1,25-dihydroxy vitamin D3 plus dexamethasone, the TLR2 ligand Pam3 CSK4, as well as a synthetic TLR4 ligand (DL8) were all identified as potent inducers of IL10 (with or without interferon γ) in naïve T lymphocytes. When administered together with OVA, all these Th1/Treg adjuvants enhance SLIT efficacy in mice by reducing both airways hyperresponsiveness (AHR), and established Th2 responses. VitD3/dexamethasone administered sublingually induces $Foxp3^+$ regulatory T cells. *L. plantarum* elicits a strong proliferation of OVA-specific T cells in cervical lymph nodes, whereas Pam3CSK4 inhibits such a T cell priming.
**Conclusions:** Several IL10 and IFNγ-inducing adjuvants enhance the efficacy of sublingual vaccines via distinct mechanisms. Such adjuvants should allow to reduce the allergen dose and simplify immunization schemes.

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**HLA-classII/peptide-TCR interactions of the single immunodominant T cell epitope of Art v 1, the major mugwort pollen allergen**

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More than 95% of mugwort-pollen allergic individuals are sensitized to Art v 1, the sole major allergen in mugwort pollen. In contrast to other major allergens, Art v 1 contains only one single immunodominant T cell epitope within Art v $1_{25\text{-}36}$ (KCIEWEKAQHGA), which is restricted by HLA-DR molecules. Immunoreactivity to this epitope is highly associated with the expression of HLA-DR1. The aim of this study was to investigate the basis of the unusual immunodominance of Art v $1_{25\text{-}36}$.
Using transfected cells expressing HLA-DRB1*0101 and HLA-DRA1*0101 in antigen presentation assays we formally showed that DR1 acts as restriction element for Art v $1_{25\text{-}36}$-specific T cell responses. Binding of Art v $1_{25\text{-}36}$ to HLA-DR-molecules was assessed in competition assays. Binding affinity of Art v $1_{25\text{-}36}$ was high for HLA-DR1 and $I_{27}$ was identified as the most probable anchor residue interacting with DR molecules in pocket P1. In addition, Art v $1_{25\text{-}36}$ bound with high affinity to isolated HLA-DR molecules typed as -DRB1*0301 and *0401, moderately to -DRB1*1301 and -DRB5*0101 and weakly to -DRB1*1101 and *1501. T cell activation was also inducible by Art v $1_{25\text{-}36}$-loaded APC expressing HLA-molecules other than DR1 indicating a certain degeneracy of peptide-binding and promiscuity of TCR-recognition. Specific binding of HLA-DRB1*0101-tetramers containing Art v $1_{19\text{-}36}$ allowed the identification of Art v $1_{25\text{-}36}$-specific T cells by flow cytometry.
In conclusion, the uniform T cell response to Art v 1 and its association with expression of HLA-DR1 is explained by the preferential binding of its immunodominant peptide to this molecule. This feature immensely facilitates future investigations of defined allergen–specific T cells by MHC classII/peptide tetramers.

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**Effects of corticosteroids on mucosal tolerance and on the development of human T cell subsets**

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**Background:** We have shown that systemic treatment with dexamethasone during the development of mucosal tolerance eliminated the generation of IL-10-producing $T_{Reg}$ cells and thus inhibited mucosal tolerance.
**Aims:** First aim was to determine whether the inhibition of respiratory tolerance is a class effect of systemically applied corticosteroids. We applied corticosteroid substances other than dexamethasone and analyzed for their capacity to inhibit respiratory tolerance.
Second aim was to analyze whether the route of delivery of corticosteroids influences their effects on respiratory tolerance. We compared the effects of inhalative versus systemic applications of steroid substances.
Our third aim was to demonstrate the effects of corticosteroids on the development of human T cell subsets (Th1, Th2, $T_{Reg}$, Th17).
**Methods:** Mice were exposed to respiratory ovalbumine to induce tolerance. Corticosteroids were applied either systemically or by inhalation. Mice were sensitized systemically with ovalbumine, and T cell proliferation and cytokine release was assessed. Regarding the generation of human T cell subsets, we established polarizing cell culture conditions to generate human Th1, Th2, $T_{Reg}$ or Th17 cells in vitro. We analyzed the effects of corticosteroids in the cultures by the expression of cytokines or of specific transcription factors.
**Results:** Systemic application of dexamethasone, prednisolone or methylprednisolone inhibited the development of T cell tolerance in vivo. In contrast, inhalative treatment with corticosteroids appeared to have no effect on the generation of $T_{Reg}$ cells and mucosal tolerance. The results of the experiments regarding the generation of human T cell subsets are pending and will be presented during the conference.
**Conclusions:** It appears to be a class effect of systemically applied corticosteroids to inhibit the development of $T_{Reg}$ cells and mucosal tolerance. The route of corticosteroid application seems crucial, since inhalative corticosteroids showed no effects on the development of mucosal tolerance in vivo. Effects of corticosteroids on the development of various human T cell subsets will be presented and discussed.

# Abstracts

## 129

**Chronic interleukin (IL)-13 stimulation of human airway epithelial cells alters the chemokine response to rhinovirus (RV)16**

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Viral infections, particularly those associated with RV, have been associated with asthma exacerbations, although the mechanisms behind this effect are not clear. Recent studies have suggested that the innate immune response (particularly that of Type I interferons) in asthmatic airway epithelial cells is diminished as compared to cells from control subjects WARK 2006. Whether a superimposed Th2 adaptive immune response could influence elements of the innate response to the virus has not been studied. We hypothesized that a chronic Th2 immune response would alter the chemokine response to RV16 in primary human airway epithelial cells in air-liquid interface.
Methods. Human airway epithelial cells from bronchial brushings from 2 severe asthmatic and 2 normal control subjects, were cultured at 1st passage in an air liquid interface system for 4-10 days in the presence of IL-13 (10 ng/ml) or media alone. In separate systems at day 1, 4 and 7, RV 16 (20 PFU/cell) was added to the upper chamber in the ALI system. 24 hrs later, RV was removed, media added and cells/supernatants harvested 24 hrs later (days 4, 7 or 10 of ALI). Cells and supernatants were analyzed for RV titer, while the supernatants were analyzed for MIG and eotaxin-3 by ELISA.
Results. In each subject's cells at each time period, the addition of IL-13 decreased viral titers in cell lysates (decreases ranging from 10% to 90%, with increasing effect with increased time in culture). MIG was not measurable at baseline or with IL-13 but increased with RV in all conditions. Eotaxin-3 was not detected at baseline or after RV but increased with IL-13. MIG levels following RV treatment of cells chronically stimulated with IL-13 were significantly lower (p=0.003) in asthma compared to normal cells, while eotaxin3 levels were similar between groups. These changes led to a marginally higher eotaxin3 to MIG ratio in asthmatics (p=0.1). Conclusions: IL-13 increases (rather than decreases) the clearance of RV. In asthma, this more rapid clearance is associated with a relative increase in eotaxin-3 with a loss of MIG. Whether these effects contribute to eosinophilic exacerbations of asthma requires further study.

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**Cytokine Modulation by Glutaraldehyde-Polymerized Ragweed Allergen**

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**Introduction:** While clinical benefits of specific immunotherapy (SIT) with native allergens and modified allergens are well studied, the impact of native versus modified allergens on immunoregulatory cytokine production is less well established. This study evaluates the influence of glutaraldehyde-polymerized (R-POL) and native ragweed pollen allergen on the IgE-regulatory cytokine synthesis.
**Methods:** Peripheral mononuclear cells (PBMC) were obtained from 12 allergic subjects sensitive to ragweed pollen during the pollen season. PBMC supernatants obtained after 9 days of culture were assayed for specific IgE by ImmunoCAP FEIA (Phadia). IL-4, IL-13 and gamma-IFN levels from PBMC supernatants were assessed by ELISA (Diaclon).
**Results:** In comparison to native allergen, R-POL induces decreased specific IgE-synthesis by PBMC. Incubation of PBMC with native allergen increases synthesis of all assessed cytokines, IL-4 increasing 1.5 fold, IL-13 increasing 2 fold and gamma-IFN increasing 1.35 fold. R-POL weakly decreases IL-4 synthesis by 1.2 fold ($p<0.05$), but increases the production of gamma-IFN by more than 4 fold. R-ROL had no effect on IL-13 synthesis.
**Conclusions:** The clinical efficacy of glutaraldehyde polymerized allergens may be related to increased gamma-IFN production than to a decrease in IL-4 and IL-13 production. Modified allergens may modulate of cytokine synthesis in a manner which differs from native, non-modified allergens.

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**The CH3 domain rather than the core hinge is primarily involved in IgG4 Fab arm exchange**

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**Background:** IgG4 antibodies are able to exchange Fab arms resulting in bispecific antibodies (1). Since a substantial fraction of IgG4 antibodies lacks covalent interaction in the hinge, which results in half molecules on non-reducing SDS-PAGE, the hinge was initially considered to be primarily responsible for the exchange of Fab arms.
**Objective:** The aim of this study was to elucidate the structural basis for the exchange of IgG4 Fab arms.
**Method:** Recombinant IgG1 and IgG4 to Bet v 1 and Fel d 1 were produced. IgG1 was mutated into the IgG4 direction by introducing a P228S mutation in the core hinge. Additionally, IgG1 CH3 domain swap mutants were made. The stability of the mutants was analysed by non-reducing SDS-PAGE. In the in vitro exchange reaction, anti-Betv1 was incubated with anti-Feld1 in the presence of reduced glutathione (GSH). Bispecificity was measured using a heterologous crosslinking assay.
**Results:** IgG1 mutants containing the hinge mutation showed IgG4-like heterogeneity on SDS-PAGE which indicates a partial absence of covalent interaction. This heterogeneity was not associated with exchange properties. IgG1 single mutants (P228S or CH3 swap mutation) did not exchange Fab arms under standard conditions where a GSH concentration of 0.5 mM was used. However, IgG1 double mutants (P228S in combination with CH3 swap mutation) did exchange Fab arms under these conditions. Furthermore, we found that increasing the GSH concentration to 5 mM resulted in bispecific activity of the IgG4 CH3 swap mutant, but not of the P228S mutant. Increasing the GSH concentration had only a minor effect on the IgG1 double mutant.
**Conclusions:** These results indicate that disruption of the inter-heavy chain disulfide bonds is required but not sufficient for IgG4 Fab arm exchange. Surprisingly, the CH3 domain has a critical role in facilitating the exchange process.

Reference
1. M. van der Neut Kolfschoten et al., Science 317, 1554-1557 (2007)

## 132

**Endogenous glycan ligands for siglec-8 and siglec-f**

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**Background:** Siglec-F and Siglec-8 are functional paralog inhibitory receptors on mouse and human eosinophils respectively. Both Siglecs preferentially recognize the ligand NeuAcα2–3(6-*O*-sulfo)Galβ1–4[Fucα1–3]GlcNAc (6'-sulfated sialyl Lewis X or 6'-su-sLeX), but its tissue distribution is unknown.
**Objective:** To explore tissue expression of 6'-su-sLeX and the sialyltransferases and sulfotransferase required for its expression in human and murine pulmonary tissues.
**Methods:** RT-PCR was used to detect the sulfotransferase KSGal6ST, uniquely needed for 6'-su-sLeX synthesis. Its distribution was studied using immunohistochemistry. Distribution of ligands on selected mouse and human lung tissues was studied via histochemistry using Siglec-F/8-Ig fusion proteins and Ig controls. The sialic acid binding dependency of Siglec-F-Ig fusion protein was explored using sialyltransferase (ST3Gal)-deficient mice and by pre-treating tissue sections with neuraminidase. Patterns of Siglec-F/8-Ig protein binding were compared to binding patterns of plant lectins specific for α2,3-linked or α2,6-linked sialic acids (Maakia Amurensis [MAA] and Sambucus Nigra [SNA], respectively).
**Results:** Quantitative RT-PCR detected KSGal6ST in whole mouse lung, and immunohistochemistry revealed selective epithelial staining. Using histochemistry, Siglec-F/8-Ig fusion proteins (but not controls) selectively bound

to normal airway epithelium. This binding pattern was identical to that seen with MAA, while SNA staining was non-epithelial. After neuraminidase pretreatment of the tissue sections, Siglec-F/8-Ig fusion proteins, MAA and SNA all failed to bind, indicating their sialic acid binding dependence. Epithelial staining was still seen in ST3Gal II deficient, but not in ST3Gal III deficient mouse lung tissue sections. Patterns of Siglec-F-Ig protein binding and MAA binding were similar in normal and murine Th2-inflamed lungs, suggesting that expression of these glycans is not altered under these conditions. Siglec-F-Ig fusion protein binding was inhibited by pretreatment of mouse lung tissue sections with MAA lectin, but not SNA lectin, further suggesting that the epithelial glycan ligand recognized by Siglec-F-Ig fusion protein contains α2,3-linked sialic acids.
**Conclusion:** α2,3-linked sialic acid-containing Siglec-F/8 ligands, and enzymes required for 6'-su-sLeX synthesis (e.g., KSGal6ST and ST3Gal III) are constitutively expressed in lung epithelium. Purification and identification of the Siglec-F/8 ligand is ongoing. Eosinophils entering the lung may have their survival shortened by encountering these endogenous sialoside ligands.

## 133
### The role of IL-25 in airways inflammation and remodeling in asthma

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Atopic asthma is a Th2 cell-mediated airway disease characterised by chronic airways inflammation and airway remodelling including lung fibrosis and angiogenesis. IL-25 (IL-17E) is newly discovered Th2-type cytokine. Several reports suggest that IL-25 participates in allergic inflammation through initiating and enlarging Th2-type responses. However, expression of IL-25 and IL-25R *in vivo* in asthmatics and other roles of IL-25 in asthma, including airways remodelling, are uncertain. Using specific antibodies, immunohistochemistry and image analysis, we measured the expression of IL-25 and IL-25R in bronchial biopsies obtained from atopic asthmatics (n = 13) and controls (n=12). In situ ELISA was employed to detect IL-25R protein in cultured human lung fibroblast cells. Cell proliferation was analyzed by MTT assay. The effect of IL-25 on angiogenesis *in vitro* was evaluated by assessing the occurrence of microvessel structures in a co-culture of primary human endothelial cells (HUVEC) and human fibroblast cells. The results showed that the numbers of IL-25 and IL-25R immunoreactive cells were significantly elevated in bronchial mucosa of asthmatics as compared controls ($p<0.003$). $FEV_1$ was negatively correlated with the numbers of IL-25$^+$ cells (r=-0596, p=0.031). IL-25R was constitutively expressed on fibroblasts and further upregulated by TNF-α. IL-25 significantly increased proliferation of lung fibroblasts *in vitro*. The *in vitro* angiogenesis assay showed IL-25 induced, concentration-dependent increases in the numbers, length and area of microvessel structures. Our data suggest that IL-25 is over expressed in asthmatic airways and plays a role in airways remodelling through its actions on fibroblasts and possibly endothelial cells.

## 134
### Mechanisms of protection from allergies early in life

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**Background:** One microbial component which was detected in abundant quantities in these rural environments was *Acinetobacter lwoffii,* a gram-negative apathogenic strain.
**Aim and Methods:** To investigate a cause-effect relationship between maternal *Acinetobacter* exposure and allergy protection in the offspring an established mouse model of experimental asthma was employed.
**Results:** Maternal *A. lwoffii* exposure prevented the development of airway inflammation and goblet cell metaplasia in the offspring to a large extent. Also, airway hyperresponsiveness was significantly normalized. These effects were accompanied by a marked upregulation of TLR-4 mRNA expression together with elevated TNF-α mRNA levels in placental tissue. In young mice the strongest protective effect of prenatal *A. lwoffii* exposure was observed following OVA sensitization with strong augmentation in TNF-α and IFN-γ production together with persistent TH-2 cytokine suppression. This effect could be linked for the first time to the bacterium *Acinetobacter lwoffii*.
**Conclusion:** These data demonstrate that prenatal early life microbial exposure has a marked impact on programming immuno-responses in early life and contributes to the prevention of respiratory allergies.

## 135
### Receptor revision of B cells in the nasal mucosa contributes to the immunopathogenesis of allergic disease

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**Background:** We have investigated the possible role of receptor revision in the generation of IgE antibodies in allergic disease. Receptor revision is defined as the reactivation of V(D)J recombination to alter the antigen specificity encoded by heavy- and light-chain immunoglobulin genes in B cells. Receptor revision requires the activity of proteins encoded by the recombinant activation genes (*RAG1* and *RAG2*). This question is important because IgE mediates the allergic response in allergy and asthma and it is not known why some individuals are sensitised to particular allergens and others are not.
**Hypothesis:** The inflammatory environment of the nasal mucosa in allergic rhinitis subjects results in the re-expression of *RAG1* and *RAG2* genes and receptor revision, whereby the antigen specificities of the immunoglobulins produced by the resident B cells are changed, leading to the local generation of new allergen specificities.
**Methods**: Biopsies were taken from the inferior turbinate of six grass pollen (GP)–sensitive subjects in-season, five subjects out-of-season and four non-allergic normal controls. We have isolated RNA from the biopsies and used RT-PCR to detect mRNA for the RAG1 and RAG2 proteins. The study is performed with the approval of the Royal Brompton Hospital Ethics Committee and the patients' written informed consent.
**Results:** RAG1 mRNA expression was detected in 6/6 GP-sensitive subjects biopsied in-season, 3/5 subjects biopsied out of GP season and 2/4 normal controls. RAG2 mRNA expression was observed in 5/6 biopsies from GP-sensitive subjects taken during the GP season, 2/5 GP-sensitive subjects biopsied out-of-season and 4/4 normal controls.
**Conclusions:** We have obtained evidence that the nasal mucosa could be a site for receptor revision, as revealed by persistent RAG mRNA expression. This strongly suggests that receptor revision of immunoglobulin genes may occur in the tissue and lead to the generation of new IgE antigen-receptor specificities, which may play a role in the immunopathogenesis of allergic disease.

## 136
### B cells precursors exist in the lung after allergen exposure in a murine model of allergic airway inflammation

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**Introduction:** B cells differentiate in the bone marrow (BM) from haematopoietic stem cells through pre-pro-B, pre-B, pro-B cells and immature B cells when they leave BM. Recent data suggest that allergy is a systemic inflammation where overproduced inflammatory cells, i.e eosinophils, move early as progenitors to the site of inflammation. However, it is not clear if this also apply for B cells. The aim of this study was to determine if early B cell precursors move from the BM to the lung after airway allergen exposure.
**Methods:** Ovalbumin (OVA)-sensitized BALB/c mice were exposed intranasally to OVA or PBS on five consecutive days. Bromodeoxyuridine (BrdU) was given during exposure to label newly produced cells. BM and lung tissue were taken 24 hours after the final allergen exposure. B220 (B cells), CD43, PB-1 and BrdU in different combinations B220+/CD43+/BrdU ( pre-pro till pre-B) and B220+/PB-1+/BrdU (pre-B) positive cells were determined by Flow Cytometry.

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**Results:** Allergen exposure increases the total cell number in the lung as well as the total number of B220+ cells. There was an increase in the number of B220+CD43+ (OVA; 0.49 ± 0.16 vs. PBS; 0.16 ± 0.05 x $10^6$; p<0.05) and B220+/PB-1+ (OVA; 0.1 ± 0.01 vs. PBS; 0.04 ± 0.17 x $10^6$; p<0.05). Additionally in above populations the relative number of newly produced; (BrdU+) were increased after allergen exposure.
In the BM there was an increase of the expression of B cell precursors. B220+CD43+ (OVA; 77.5 ± 2.27 vs. PBS; 39.88 ± 1.07, p<0.05; % of total $B220^+$) and B220+PB-1+ (OVA; 35.33 ± 1.2 vs. PBS; 31.2 ± 0.7, p<0.05; % of total $B220^+$).
**Conclusion:** B cell precursors were detected in lung both already after sensitization and further increased after allergen exposure. The majority belonged to the earlier stage of B cells precursors. There was an increase in their proportion that had been produced after allergen exposure, proposing an active procedure. Those new data together with the increase generation of B cell precursors in the BM suggest that during allergic inflammation there is an early motivation of B cells into the active site of exposure.

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### Human enterocytes express FcεRI: effector cell candidates in IgE-mediated intestinal disorders

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**Background:** In the intestinal mucosa IgE antibodies play a paramount role in the pathogenesis of various disorders. For contribution to symptoms or protection from diseases intestinal mast cells, eosinophils or antigen presenting cells acquire effector potency through specific IgE binding via the high affinity receptor FcεRI. However, also FcεRI-expressing enterocytes might, in concert with IgE, contribute to pathologies. Therefore, in the current study we aimed to investigate the expression of FcεRI on human intestinal epithelium. **Methods & results:** By immunohistochemistry and immunofluorescence the presence of FcεRI α-chain was examined in paraffin-embedded intestinal tissue sections from patients with colon cancer, gastrointestinal inflammatory disorders, or without pathologies of the gastrointestinal tract. A FcεRI α-chain positive staining was detected in 7 out of 11 (7/11) colon cancer patients and 4/11 patients with inflammation in the small intestine as well as in the colonic mucosa. The most prominent FcεRI-expressing cells were identified as Paneth cells due to co-localization of FcεRI expression with defensin 5, a Paneth cell marker. The specific IgE binding capability of the identified receptor was confirmed in situ, as only fluorescence-labeled IgE but not IgG bound upon passive sensitization. Interestingly, immunocytochemical staining and Western blotting of human intestinal cell lines CaCo2 (TC7) and HCT8 indicated a functional FcεRI (α-chain and γ-chain) only in undifferentiated, subconfluently grown TC7 and HCT8 cells. **Conclusions:** Our data revealed for the first time a differentiation-associated expression of FcεRI in human intestinal epithelial cells, which was especially pronounced in the adjacent mucosa of colon tumors.
**Funding:** This work was supported by OeNB Jubiläumsfond 11375, a Hans and Blanca Moser Fund and FWF PA-18238-B13.

### 138
### IgG4 antibodies do not prevent the subsequent development of IgE antibodies. A study in a cohort of apprentice laboratory animal workers

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**Background**: IgG4 antibody production is a TH2-dependent process. However, IgG4 responses often occur in the absence of IgE antibody production ("modified TH2 response). Such an IgG4 response has been claimed to reflect a state of antigen-specific tolerance.
**Objective:** Establish whether pre-existing IgG4 antibody prevents the development of IgE antibodies.
**Methods:** The dynamics of IgE- and IgG4 anti-rat allergen responses were investigated in a cohort of apprentice laboratory animal workers with, at inclusion, a negative rat allergen skin test and less than 12 months experience with animal work (n=110). In addition to serology, skin tests, allergen exposure and in-vitro allergen-induced cytokine profiles were monitored.
**Results:** After 2 years follow up a positive SPT for rat allergens was found in 16%. An exposure – response relationship was found (p=0.011). No significant relationships were found between exposure levels and specific IgG4. No significant changes were found in IgG4 levels over the two year follow up. Levels of specific IgG4 at inclusion were comparable in the group that developed sensitization compared to the group that did not develop sensitization. However, an increase in IgG4 levels was found in LAW developing sensitization between the last visit before sensitization and the first visit after sensitization. IL-4 responses to rat allergen were measured for the 18 SPT positive LAW and 18 controls matched on atopic status. IgG4 levels of the SPT positive and negative LAW were comparable. IL-4 spots correlate with levels of rat specific IgE (rho: 0.736, p<0.001) but not with levels of rat specific IgG4.
**Conclusion:** No protective effect of IgG4 on the development of rat specific sensitization was found.

### 139
### T cell-independence of secondary IgE responses in allergy

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Allergic sensitization to environmental allergens is characterized by a TH2- and cytokine-dependent development of an allergen-specific IgE response. In already sensitized patients allergen contact induces an increase of allergen-specific IgE levels and sensitivity to allergens, but the mechanisms underlying the secondary IgE responses are poorly understood. We were interested to study the contribution of TH cells to the maintenance and boosting of secondary IgE responses. In a murine model of grass pollen allergy we investigated the effects of co-stimulation blockade on primary sensitization and secondary IgE responses. Co-stimulation blockade at the time of sensitization prevented the development of allergen-specific IgE responses compared to untreated mice. However, co-stimulation blockade had no influence on established IgE responses in sensitized mice. Allergen-specific T cell activation was suppressed in mice by early as well as late co-stimulation blockade suggesting that IgE-responses in sensitized mice are independent of T cell help. These observations are supported by data from allergic patients. A study performed in atopic dermatitis patients receiving systemic cyclosporin A treatment showed a reduction of T cell-mediated skin symptoms. However, cyclosporin A had no effect on the boosts of secondary IgE responses induced by seasonal allergen exposure. Additional evidence comes from the analysis of patients suffering from AIDS, who displayed an IgE reactivity and skin sensitivity to environmental allergens despite low T cell counts. The finding that secondary allergen-specific IgE responses seem to be independent of T cell help are important for understanding the pathomechanisms of allergic diseases and the development of immunotherapy strategies.
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### Keratinocytes as immunoregulators during inflammatory skin reactions

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**Background:** Allergic contact dermatitis (ACD) and atopic eczema (AE) are eczematous skin diseases in which T cells are directly involved in the induction of keratinocyte death. However, almost nothing is known how keratinocytes

influence T cell activity. The aim of this study was to investigate the outcome of keratinocyte-T cell interactions with focus on T cell functions such as proliferation and cytokine release in an antigen-specific *in vitro* model of ACD to Nickel and acute AE.
**Methods***:* T cell clones were generated from PBMC of *Phleum pratense* - (n=2) and non-atopic Nickel sensitised patients (n=5) and incubated with autologous antigen-presenting cells (APC) together with autologous primary human keratinocytes. Crosstalk of keratinocytes and T cells was analysed with respect to T cell proliferation and cytokine production.
**Results:** Coincubation of keratinocytes, Nickel-specific T cells and APC revealed that keratinocytes variably influence the proliferation of T cells with 84% T cell clones (n=27) being blocked and 16% (n=5) being induced in antigen-specific proliferating capacity. Notably, production of the cytokines IL-4 and IL-10 was regulated independently from the proliferation showing always a reduction of IL-4 and IL-10 release. IFN-γ was not constantly regulated. Coincubation experiments with Phl p 5-specific T cell clones and autologous keratinocytes show similar results with T cells being blocked in proliferation and IL-4 and IL-10 cytokine release in the presence of keratinocytes. In contrast to Nickel-specific T cells, IFN-γ is significantly induced in Phl p 5-specific clones in the presence of keratinocytes. Neither differentiation status nor preincubation of keratinocytes with IFN-γ impact the immunomodulatory effect of keratinocytes. The observed T-cell-keratinocyte crosstalk was cell-contact independent since keratinocyte cell culture supernatant had comparable impact on T cell function as keratinocytes themselves. Fixation of keratinocytes with paraformaldehyde (PFA) abrogated their immune-modulating effect on T-cells.
**Conclusion:** Our results suggest that keratinocytes can actively influence the effector phase of eczematous reactions by modulating the effector functions of skin infiltrating T cells. The immune-modulating effect on T cell proliferation and cytokine production seems to be mediated by soluble factors. Presumably, keratinocytes act as modulators of T cell function and skin micromilieu.

## 141

**Exploring allergic responses in the skin of humans *in vivo* using laser imaging, dermal microdialysis and skin biopsies**

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In previous studies we have used scanning laser Doppler imaging, microdialysis and immunocytochemistry to explore the mediator and cellular mechanisms of the dermal allergic response. The early phase response was accompanied by rises in histamine and $PGD_2$. No leukotrienes were detectable even though at 8 hours after allergen provocation there were increased numbers of eosinophils expressing 5-LO and FLAP.
We have now extended these studies to assess the time course of cytokine generation from discrete sites within the skin following allergen injection using two microdialysis probes, one close to the point of allergen injection and the other 1 cm away but within the area of the late phase induration. At baseline, microdialysate contained low levels of IL-1α, IL-5, IL-8, IL-12, GM-CSF and TNFα. At control sites (wounding reaction) IL-6 and IL-8 were increased at 3 and 6 hours. Allergen increased TNFα levels in 3/11 individuals within 30 minutes at the injection site. Levels of IL-6 and IL-8 rose rapidly and were significantly greater ($P < 0.05$) than control at 3 and 6 hours at both injection and distant sites. Skin biopsies taken at 6 hours showed increased expression of adhesion molecules and recruitment of neutrophils and eosinophils only at the allergen injection site even though there were high levels of IL-6 and IL-8 at the site 1 cm away. This suggests a complex relationship between cytokine generation and cellular events in allergic inflammation.
We have also investigated the development of the histamine-induced neurogenic flare response using a full-field laser profusion imager (FLPI™, Moor Instruments, Axminster, UK) which may capture up to 25 images per second of 40,000 pixels per $cm^2$ over an area of 8 $cm^2$. The results showed a decreasing rate of increase of blood flux with increasing distance from the injection site. Within 5 mm of histamine injection, maximal blood flux was reached within 20 seconds whereas 15 mm away there was an irregular increase over 150 seconds.
In conclusion, microdialysis used in conjunction with laser imaging and skin biopsies provides novel information about the mechanisms of dermal inflammation *in vivo*.

## 142

**Intracellular cAMP-elevating agents enhance thymic stromal lymphopoietin production by airway tissue-derived cells**

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**Rationale:** Thymic stromal lymphopoietin (TSLP) as well as intracellular cAMP-elevating agents such as forskolin activate dendritic cells to induce development of Th2 cells from naïve T cells. One of cAMP-elevating agents, $\beta_2$-adrenoceptor agonist widely used for treating asthma attacks also enhances development of Th2 cells and increases airway hyperresponsiveness to spasmogens. Here, we have tested whether or not cAMP-elevating agents affect TSLP production by airway tissue-dwelling cells *in vitro*.
**Methods:** We used dibutyryl cAMP (10 μM), 8-bromo-cAMP (10 μM), forskolin (1 μM), formeterol ($10^{-12}$–$10^{-8}$M), salmeterol ($10^{-12}$–$10^{-8}$M), salbutamol ($10^{-12}$–$10^{-8}$M) as intracellular cAMP-elevating agents. Human bronchial smooth muscle cells (BSMC), human lung blood microvascular endothelial cells of lung (HMVEC-LBL), human lung-derived fibroblasts (NHLF) and human bronchial epithelial cells (NHBE) were used for TSLP induction (ELISA and real-time PCR) *in vitro* assay. These cells were plated at $10^5$ cells/ml appropriate media on collagen-coated plates for 48 hr and then stimulated with poly I:C (20ng/ml), TNF-α (10ng/ml) and IL-4 (10ng/ml) individually or in combination for 6 hr (real time-PCR) or 48 hr (ELISA) with or without cAMP-elevating agents.
**Results:** A combination of IL-4 and TNF-α most strongly induced TSLP production (up to 200 pg/ml) by all the cell types expect for the effect of IL-4 and poly I:C on NHBE. $\beta_2$-agonists significantly enhanced (up to 3-fold compared to the combination of IL-4 and TNF-α) the cytokine-induced TSLP production by these primary airway tissue-derived cells even at $<10^{-10}$ M (formeterol and salmeterol). Forskolin, dibutyryl cAMP and 8-bromo-cAMP also significantly enhanced the TSLP production (up to 5-fold). Similar enhancing effect of the cAMP-elevating agents were observed when mRNA was examined using real-time PCR.
**Conclusion:** Intracellular cAMP agents may act as Th2 adjuvants, at least in part, through the production of TSLP by tissue-dwelling cell types.

## 143

**Adoptive transfer of Th clone conferred asthma phenotypes including airway obstruction**

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Helper T (Th) cells and Th2 cytokines have been strongly implicated in eosinophilic inflammation, mucous hypersecretion, airway hyperresponsiveness (AHR) and remodeling. Role of Th cells in airway obstruction is yet unclear.
A panel of murine ovalbumin (OVA) specific Th clones were derived 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 intranasal or inhalation challenge with OVA, airway eosinophilia and bronchial responsiveness to methacholine were analyzed. Several Th clones conferred both BALF eosinophilia and AHR, and others conferred either one of them, suggesting that AHR is not necessarily dependent on airway eosinophilia. A Th clone T6-2 conferred airway eosinophilia which was detectable 48 hr after antigen challenge. Mice transferred with T6-2 and challenged with OVA showed airway obstruction in terms of BUXCO unrestrained body plethysmography before inhalation of methacholine, whereas mice transferred with other eosinophilia- and AHR-inducing Th clones exhibited airway obstruction only after inhalation of methacholine. The finding was confirmed by measuring airway resistance under restrained, anesthetized, and intubated conditions. The airway obstruction was detectable 6 hr after antigen challenge, at which time period no cellular infiltration was detectable in BALF. Moreover, airway obstruction was induced not only by OVA but also by OVA peptide 323-339, the T cell epitope. Our results clearly demonstrated that activation of Th cells resulted in airway obstruction besides induction of inflammation, mucous hyperplasia, and AHR.

# Abstracts

## 144

**Establishment of severe allergic asthma and dermatitis models caused by repeated application of Dermatophagoides farinae**

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Dermatophagoides farinae (Der. f) are known to be a common environmental allergen causing allergic asthma and atopic dermatitis. The aims of this study is to establish a pharmacological model of allergic diseases caused by Der. f. Regarding asthma model, mice were instilled with crude extract of Der. f without additional adjuvant into the trachea eight times. After last allergen instillation, the airway responsiveness to acetylcholine was measured, and bronchoalveolar lavage and histopathological examination were carried out. The instillation of the allergen induced airway hyperresponsiveness, the accumulation of inflammatory cells and increases in the levels of Th2 cytokines and transforming growth factor β production in the bronchoalveolar lavage fluid. The number of goblet cells in the epithelium and extend of the fibrotic area beneath the basement membrane were also increased in the histopathological study. In contrast, the defect of IL-4/ IL-13 signaling through IL-4 receptor α chain completely abrogated all these responses. Furthermore, fluticasone propionate showed significant inhibition or an inhibitory tendency of these changes. These findings demonstrate that this model is suitable for investigating the pharmacology of Th2 dependent allergic airway asthma.
Next, we tried to establish a mouse model for atopic dermatitis. Crude extract of Der. f was painted 5 times repeatedly at an interval of 7 days onto the ear of NC/ Nga mice with simultaneous tape-stripping. Apparent biphasic ear swelling and histopathological changes similar to atopic dermatitis were observed after allergen application. The ear swelling was accompanied by increased serum IgE, increased expression of IL-4 mRNA and decreased expression of IFN-γ m RNA in cervical lymph nodes and ears. These changes are inhibited by the administration of prednisolone. Above results indicate that this model is also suitable for pharmacological investigation of atopic dermatitis.
In conclusion, we have established a stuitable pharmacological model for allergic asthma and dermatitis by repeated application of Der. f to the mouse.

## 145

**Immunoglobulin free light chains are present in chronic inflammatory diseases: Studies in allergic and non-allergic rhinitis, multiple sclerosis and rheumatoid arthritis**

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In previous work we have shown that immunoglobulin free light chains (FLC) can elicit IgE-independent hypersensitivity responses. In murine models for contact sensitivity and non-atopic asthma, FLC were found to play a crucial role in eliciting clinical symptoms of disease. It has been shown earlier that various chronic inflammatory diseases are accompanied with the presence of increased FLC concentrations. In this study we focused on the presence and localization of FLC in tissue biopsies from rhinitis, multiple sclerosis (MS) and rheumatoid arthritis (RA) patients.
**Methods:** Specimen from brain tissue of healthy donors and MS patients (Dutch Brain Bank), synovial tissue from RA patients (donated by D. Woolley, UK) and nasal mucosa from allergic and non-allergic rhinitic mucosa and normal control mucosa were stained with antibodies specific for lambda and kappa immunoglobulin free light chains, and other markers such as mast cell tryptase, CD20 (B cells), CD68 (macrophages), CD138 (plasma cells). From nasal mucosa samples, immunoglobulin free light chain immunoreactive cells were isolated with laser microdissection and further analyzed with RT-PCR for expression of tryptase or CD138.
**Results:** Staining for FLC was detected in (chronic) active MS lesions. No staining was observed in chronic inactive MS lesions, gray matter lesions, normal appearing white matter or control brain tissue. Staining was always cell-associated, indicative of surface-bound or intracellular light chains. Positive cells were detected in parenchyma, small vessel walls and perivascular cuffs. Tryptase-positive cells were detected in MS (chronic) active MS lesions, whereas no staining was observed in chronic inactive MS lesions, gray matter lesions, normal appearing white matter and control brain tissue. Preliminary studies showed that both kappa and lambda-positive cells were detected in synovial tissue from RA patients. Analysis of serum FLC demonstrated that FLC concentrations in RA patients are greatly increased when compared to serum from healthy subjects. FLC expressing cells were significantly increased in allergic and non-allergic rhinitic mucosa compared to normal tissue. Microdissected FLC-immunureactive cells were confirmed to be a mix of mast cells and plasma cells.
**Conclusions:** Immunoglobulin free light chains are present in foci of inflammation in brain tissue from MS patients, synovial tissue from RA patients and allergic and non-allergic rhinitic mucosa. Our study supports the proposal that immunoglobulin free light chains can be involved in the induction or propagation of chronic inflammation.

## 146

**Mucosal allergen challenge induces therapy-resistant, long-lived IgE plasma cells**

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Allergen-specific IgE antibodies are responsible for the pathogenesis of type I hyperreactivities. In allergic patients, specific IgE remains present even without allergen-contact for long periods of time. Seasonal allergen exposure leads to the onset of clinical symptoms, but is also known to trigger IgE responses of antibody-secreting plasma cells (PC). Most PC are short-lived. However, a fraction of ASC migrates to the bone marrow, becomes long-lived and continues to secrete either protective or pathological antibodies for extended periods. Whether mucosal aerosol challenge induces short-lived allergen-specific IgE-ASC which maintain chronic inflammation through an ongoing allergen-activation or become long–lived and survive in inflamed tissues such as the lung or elsewhere was not clear until now.
We analyzed the origin and maintenance of allergen-specific IgE, IgA and IgG PC in an ovalbumin-dependent murine model of allergic asthma by ELISA, ELISPOT and fluorescence microscopy. Balb/C mice were systemically sensitized with OVA-alum and subsequently challenged with OVA-aerosol.
We show, that mucosal aerosol treatment potentiates the systemic IgE immune response with variable lifespans. Continuous mucosal aersosol-challenge induces OVA-specific PC of all isotypes in the lungs. Termination of the aerosol-treatment leads to a local loss of PC in the lungs but survival of OVA-specific PC in spleen and preferentially in the bone marrow maintaining systemic OVA-specific Ig titers without further antigen contact. These PC survive systemic cytostatic treatment and add up to the pool of long-lived PC. This suggests, that continuous allergen inhalation generates an inflammatory milieu in the lungs which may harbour PC. Our data validate the unique role of long-lived, allergen-specific IgE-secreting PC as prime therapeutic target of type I allergy, a target so far unrecognized.

## 147

**Monitoring cat allergy in childhood by using the major cat allergen, rFel d 1**

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**Background**: Domestic cat is one of the most significant sources of indoor allergens world-wide causing asthma. We have recently reported that increased IgE levels to the major cat allergen, rFel d 1, are a risk factor for asthma in cat allergic children. In order to predict and prevent the development of allergic

asthma, detection of early cat sensitisation is important. We here elucidated the usefulness of rFel d 1 in the development of cat allergy in childhood.
**Methods:** 144 children from the BAMSE birth cohort, Stockholm, Sweden, with asthma or rhinoconjunctivitis in contact with cat or where such symptoms were suspected, were selected. Blood samples taken at both 4 and 8 years of age were analysed for allergen-specific IgE to cat dander extract (CDE) and recombinant (r) Fel d 1. IgE antibodies to CDE were measured with the ImmunoCAP System (cut-off limit 0.35 $kU_A/L$) and to rFel d 1 by ELISA (cut-off limit 0.037 $kU_A/L$).
**Results:** Among children being certain of symptoms at 4 years of age, 25/33, had detectable IgE levels to both rFel d 1 and CDE. At the same age, 14/42 of those suspecting symptoms at 4 years had IgE antibodies to rFel d 1, but only 9/42 to CDE. Among children reporting symptoms for the first time after age 4, 60/69 were detected with rFel d 1 and 57/69 with CDE at age 8. In latter group, 33/69 had IgE to rFel d 1 already at 4 years, while 26/69 to CDE. When evaluating rFel d 1 levels over time in this group, a significant increase from 4 to 8 years was seen ($p<0.0001$), even when only children with IgE to rFel d 1 already at 4 years were included ($p<0.001$).
**Conclusions:** We show that cat sensitisation can be detected several years before symptoms to cat are developed by using a sensitive rFel d 1 assay. Furthermore, we demonstrated that IgE antibodies to rFel d 1 at age 4 are associated with symptoms to cat later in childhood.

## 148

### Lipid transfer protein (LTP) and profilin have opposite effects in the clinical reactivity to apple in allergic patients from Spain

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**Objective**: To study the factors associated with the clinical presentation of apple allergy and the outcome of double-blind, placebo–controlled food challenges (DBPCFC) with apple in the Spanish population.
**Methods**: In 99 patients who reported immediate reactions and had positive prick-prick tests with fresh apple, a careful medical history was collected, the IgE responses to the apple allergens Mal d 1-2-3-4 were analysed by RAST and reactivity to apple was assessed by DBPCFC. The clinical presentation of apple allergy as reported in the medical history was classified into local or systemic, and the outcome of the DBPCFC with apple into positive or negative (placebo reactors were excluded). By means of logistic regression the factors associated with a positive DBPCFC and with reported systemic reactions were analysed. The following variables were included: age at study and at first reaction with apple, sex, pollen allergy, pollen asthma, duration of apple allergy, and RAST to Mal d 1-2-3-4.
**Results**: Only the IgE responses to Mal d 3 (LTP) and Mal d 4 (profilin) were significantly associated with a positive DBPCFC. The probability of a positive DBPCFC was similar in the presence of specific IgE to both Mal d 3 and 4 (91.7%) or to any of them separately (85.7% if negative Mal d 3 and positive Mal d 4; 90.3% if positive Mal d 3 and negative Mal d 4). In the logistic model the only variables significantly associated with systemic reactions in the clinical presentation were the IgE responses to Mal d 3 (OR 7.02, 95%CI 1.64-30.00, p= 0.003) and Mal d 4 (OR 0.18, 95%CI 0.06-0.49, p< 0.001), and pollen asthma (OR 0.23, 95%CI 0.08-0.65, p= 0.004).
**Conclusion**: Apple LTP and profilin are clinically relevant allergens in Spanish patients allergic to apple, although with opposite effects. Sensitisation to apple LTP is a risk factor for systemic reactions, whereas the IgE response to apple profilin reduces that risk. The presence of pollen related asthma also reduces the risk of having systemic reactions with apple.

## 149

### Component Resolved Diagnostics in apple and pear allergy across Europe

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**Background:** Rosaceae fruits are among the most common causes of food allergic reactions throughout Europe. Apple is known for its allergenic association to birch pollen but in areas with no or few birch trees, no clear-cut pollen association is known. Pear contains a complement of allergens similar to that of apple but is less known as a cause of food allergic reactions.
**Purpose:** To study symptoms and sensitization patterns in apple and pear allergy in the central and south of Europe.
**Methods:** A total of 170 subjects from Germany (DE), Switzerland (CH) and Spain (ES) were enrolled in the study. Apple and pear allergic patients (n=80) were included based on positive case history and open food challenge (OFC) with fresh fruit. Sixty atopic controls were included based on negative case history and OFC, as well as 30 nonatopic controls with no indication of allergic disease. Specific IgE to a range of food and pollen allergen sources and to recombinant apple and pear allergens was measured using ImmunoCAP. SPT was performed with pollen and food extracts and with fresh fruit.
**Results:** Oral symptoms to apple and/or pear were the most common, reported by all but 2 of all food allergic subjects. Additional symptoms, indicating systemic or severe reactions, occurred in 14 of 22 (64%) Spanish and 16 of 58 (28%) central European subjects and was more than twice as common to apple as to pear. However, symptoms to pear in the Spanish subjects appeared more severe than symptoms to apple in central Europeans. Apart from one case (CH, pear), no reactions to cooked fruit were recorded. Sensitization to birch and hazel pollen was present in all central European subjects and to grass pollen in 78%. Among the Spanish, sensitization was most common to plane (36%), mugwort (32%), grass (32%) and olive (23%) pollen. One third of the Spanish subjects were not sensitized to any of the 8 pollens tested. Quantitative measurements of IgE responses to rMal d 1, 3 and 4 from apple and Pyr c 1, 3, 4 and 5 from pear were performed in all subjects and will be presented.

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### Component resolve diagnosis in every day allergy practice

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**Background**: Proper treatment of allergic patients depends on accuracy in diagnostic procedure.
**Hypothesis**: IgE determination to purified allergens can improve pollen allergic patient diagnosis and modify in some cases therapeutic decisions.
**Material and Methods**: Thirty-four patients suffering from seasonal respiratory complaints were evaluated in a traditional way by anamnesis, skin prick test and serum specific IgE against complete extracts (CAP System). Specific IgE against purified molecules were also determined (Centaur System): Art v 1, Bet v 1, Cup s 1, Ole e 1,Ole e 9, Par j 1, Phl p 1, Phl p 5, Pla l 1, Sal k 1, Che a 3 (polcalcin), Mal d 4 (profilin) and Pru p 3 (LTP). Two independent allergists established immunotherapy decisions before and after results of molecular diagnosis were known, according to explicit criteria.
**Results**: Taking into account only traditional diagnosis our immunotherapy decision results were as follows: 29 patients (85.3%) would receive grass pollen immunotherapy; one patient, grass and olive pollen treatment and, in the remaining 4 cases (11.8%) immunotherapy would not have been indicated. Considering besides molecular information, changes in indication or composition of immunotherapy were done in 4 patients (11.8%). Plantago immunotherapy was prescribed to 1 patient in whom vaccination had not been firstly indicated; Two grass immunotherapy treatments were changed to grass and olive in one patient and to grass and plantago in another. . Finally, just olive pollen immunotherapy was prescribed to one subject who at the first time, would have received grass and olive pollen vaccination..
**Conclusion:** Component resolved specific IgE determination could avoid panallergens-induced diagnostic mistakes and could modify therapeutic decision in 11.8% of our pollen allergic patients.

# Abstracts

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### Prediction of allergic patients' phenotypes with recombinant pollen marker allergens

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**Background:** During the last decade allergen molecules from several allergen sources have been produced by recombinant DNA technology.
**Objective:** The aim of the study was to investigate whether recombinant pollen allergens with broad and narrow cross-reactivity can be used as diagnostic tools to predict phenotypes of allergic sensitization.
**Methods:** Serum IgE reactivity to a selected panel of six recombinant birch and grass pollen allergens was measured by ELISA in pollen sensitized patients from Central Europe to define groups of patients i) with exclusive IgE reactivity to rBet v 1, ii) with exclusive IgE reactivity to major grass pollen allergens (rPhl p 1, rPhl p 2 and rPhl p 5) and iii) with IgE reactivity to cross-reactive pollen allergens (rBet v 2, rPhl p 7). Patients' clinical phenotypes were recorded. Then responses to tree, grass and weed pollen as well as plant food extracts were evaluated in vitro by CAP-FEIA and clinical sensitivities were confirmed in vivo by skin prick testing.
**Results:** IgE reactivity to the recombinant major birch pollen allergen, rBet v 1, predicted sensitization to pollen from birch, taxonomically related trees and to certain plant-derived foods. Reactivity to the recombinant timothy grass pollen allergens, rPhl p 1, rPhl p 2, rPhl p 5 indicated sensitization to pollen of grasses. Patients reacting with the highly cross-reactive allergens rPhl p 7 and/or rBet v 2 were polysensitized to pollen from unrelated trees, grasses and weeds (rPhl p 7) as well as to plant-derived food (rBet v 2).
**Conclusion:** IgE reactivity profiles to recombinant pollen allergens can be used to predict phenotypes of allergic sensitization.

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## 152

### Use of recombinant allergens for diagnosis of mite and cockroach allergy in Brazilian children

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**Background**: Recombinant allergens may provide advantages over currently available commercial extracts, and have been shown to induce positive skin tests in patients with asthma and/or rhinitis. The use of recombinant allergens for diagnostics has not been investigated in a large, less selected population.
**Methods:** A group of 531 school children 13-14 years-old, living in Southeast Brazil, was selected among 4498 children evaluated by the ISAAC questionnaire, using a 1:2 ratio of presence and absence of wheezing in the past year. Children underwent skin prick testing with commercial inhalant extracts, and recombinant allergens derived from mites (rDer p 1, r Derf 1, rDer p 2, rDer p 5, and a mixture containing all four allergens) and cockroach (rPer a 1, rPer a 7, rBla g 2, rBla g 4, rBla g 5) at 10mcg/mL concentration. Skin tests were considered positive when a wheal of equal or greater than 4mm mean diameter accompanied by erythema developed 15 minutes following allergen application.
**Results:** Presence of a positive skin test to mites, cockroach, cat and dog; to the mite recombinant mix, and to each of the recombinant allergens except rPer a 1 (reactivity 1.3%) was significantly associated with current wheezing. Kappa analysis revealed a strong concordance of results of skin tests using commercial mite extract and recombinant mite mix (index of 0.7591); and a moderate concordance using *Periplaneta americana* extract and recombinant *P. americana* allergens rPer a 1 and rPer a 7 (index of 0.4637). Among 251 children with positive skin tests to *D. pteronyssinus*, 190 (75.7%) had positive tests to the recombinant mix, and only 2/280 with negative tests to *D. pteronyssinus* presented positive reactions to the recombinant allergens. Positive reactions showed median values for wheal diameters of 7mm for both *D. pteronyssinus* commercial extract and recombinant mite mix.
**Conclusion:** Mixtures of recombinant mite allergens can be safely and effectively used for the diagnosis of mite allergic patients.

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### Cross-reactive carbohydrate determinants and *in vitro* allergy diagnostics: comparison of three conceptually different automated allergen-specific IgE-detection systems.

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Cross-reactive carbohydrate determinants (CCD) are probably the most widely distributed pan-epitopes without established clinical relevance. They seem to decrease in vitro-test specificity considerably. Is the CCD-phenomenon dependent on the detection method?
Sera of 34 insect sting allergic patients with IgE to the venom of honey bee (HB) plus yellow jacket (YJ) were investigated with CCD-allergens: bromelain (BRO), horseradish peroxidase (HRP), and natural rubber latex (NRL) in CAP FEIA (CAP) (Phadia), using a solid ImmunoCAP matrix; Immulite (IML) E 2000 (DPC Biermann), based on liquid-phase technology; and with HB and YJ extracts as well as the non-glycosylated recombinant allergens Api m 1 (HB) and Ves v 5 (YJ) (ALK-Abello) in the ADVIA-Centaur Allergy Screen Assay (Bayer HealthCare Diagnostics Division), based on a reverse sandwich architecture with monoclonal mouse-anti-human-IgE bound to paramagnetic particles in the solid-phase and capturing the sample IgE. All sera had IgE to both insect venoms in CAP, whereas it was 88% in IML, 33% in ADVIA. In IML 3/4 HB-IgE-negatives were highly positive for YJ-IgE, 1/4 was only positive for HRP- and BRO-IgE, and another one was negative for YJ-IgE. In CAP, 24/34 (71%) had IgE to BRO, 27/34 (79.4%) to HRP, 21/34 (62%) to NRL. In IML, 20/34 (59%) had IgE to BRO, 25/34 (74%) to HRP, 14/34 (41.2%) to NRL. In ADVIA, 29/33 had IgE to YJ-, 14/31 to HB-extract, 11 to both. 12/34 bound to Api m 1, 26/34 to Ves v 5, 8/34 to both. IML-results not corresponding with CAP showed accordance with ADVIA in 4/4 HB-IgE negatives, 3/4 being positive for YJ-IgE in both systems. The serum only HRP- and BRO-IgE-positive in IML had YJ-IgE in ADVIA. CCD-IgE were detected by both, CAP and IML, HRP being the dominant CCD-allergen. NRL-IgE had no clinical relevance. As in CAP the CCD-IgE-detection via BRO and HRP was comparable, these epitopes seem to be equally presented on the respective ImmunoCAPs. The differences between BRO and HRP in IML might be due to the CCD-presentation in these allergens in liquid-phase. Test specificity can be increased by parallel testing of CCD- and relevant recombinant allergens.

## 154

### Differentiating between true peanut allergy and asymptomatic sensitization to peanut using microarray and component-resolved diagnostics

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**Background**: Diagnostic tests to assess peanut sensitisation are based on natural peanut extracts which contain allergenic and non-allergenic molecules. As some of these may cross-react with pollen allergens, the prevalence of peanut sensitisation may be higher than that of true peanut allergy. Diagnostic tests which can differentiate asymptomatic sensitisation from true peanut allergy without the use of oral food challenge (OFC) would be extremely useful.
**Objectives:** To establish the prevalence of true peanut allergy amongst children

diagnosed as sensitised using standard skin tests (SPT) or sIgE measurement; and whether component-resolved diagnostics using microarray helps differentiate asymptomatic sensitization from true peanut allergy.
**Methods:** In a population-based birth cohort, we ascertained peanut sensitisation by SPT (n=920) and sIgE measurement (n=605) at age 8 years; interviewer-administered questionnaires captured the information on reactions upon peanut exposure. Amongst sensitised children, we confirmed peanut allergy by OFC (12 subjects with convincing history, sIgE≥15kUa/L and/or SPT≥8mm were considered peanut-allergic without challenging). We used open OFC amongst those consuming peanuts (n=46); otherwise, children underwent double-blind placebo-controlled OFC (n=35). We compared sensitization profile between peanut allergic and tolerant children using a capillary-flow membrane-based microarray on which we immobilized pure components from peanut (Ara h 1-3 and 8), grass (Phl p 1, 4, 5b, 7, 12) and other cross-reactive components (Bet v 1, Pru p 3 and CCD).
**Results:** We diagnosed peanut sensitisation in 14.7% (sIgE) and 5.2% (SPT) of children; 1.7% reported symptomatic peanut allergy. Of 81 children who underwent OFC, 14 reacted. The prevalence of true peanut allergy among sensitized children was 28% (95%CI 19.9%-37.8%). Children with true allergy (group enriched with 12 peanut-allergic children recruited from local hospitals) recognised major peanut allergens Ara h 1-3 significantly more commonly compared to sensitised subjects with negative OFC (90%, 100%, 86% *vs.* 50%, 44%, 25%, Ara h 1, 2 and 3 respectively); in contrast, reactivity to Phl p 4 was significantly less common (48% *vs.* 83%); $p<0.05$ (adjusted for multiple testing).
**Conclusions**: The majority of children diagnosed as peanut sensitised using standard tests do not have true peanut allergy. The component-resolved diagnostics may facilitate the diagnosis of true peanut allergy.

## 155

### Specific IgE measurements by microchips: Theoretical considerations and a comparison between the ISAC®-chip and the immunoCAP

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**Background:** The miniturization of specific IgE measurements by chip technology offers potentials in terms of component-resolved diagnosis, low serum consumption and minimal price per IgE-specificity. We aimed at studying various designs including the commercially available ISAC-chip (VBC-genomics).
**Methods:** Different in-house designs of IgE-measurements were tested and a comparison was made between the ISAC-chip (VBC-genomics) and the ImmunoCAP (Phadia), both of which were performed according to the instructions of the suppliers. Twelve patients' sera were tested in both methods. Three of these had received immuno-therapy with grass, mite and bee venom extracts, respectively. Another nine were selected based on their positive IgE tests to various allergens in the ImmunoCAP. Finally, a pool of sera (NHS) commonly used as a non-allergic control was tested on the ISAC. NHS contained sera from patients negative in the ImmunoCAP to a standard panel of ten inhalation allergens and a food panel (milk, egg, wheat, soy, peanut, codfish).
**Results:** The three sera from patients that had received immunotherapy were positive for IgE in the ISAC-chip indicating that the chip response is not sensitive to allergen specific IgG antibodies. NHS tested negative for all allergens on the chip except for a weak response to a cow's milk allergen Bos d7. Three of four patients that were positive to latex in the ImmunoCAP were shown to have reactivities to one or more of the nine individual latex allergens present on the allergen chip. Two patients CAP-positive to egg and milk extracts were also positive on the chip to egg and milk allergens. One patient class 3 positive to shrimp in the CAP-test was negative to two shrimp allergens present on the chip, which might be explained by a difference in shrimp species. Generally, the number of allergens identified on the chip and the level of specific IgE measured by CAP (class) did not correlate well. Attempts to reverse the assay design by catching anti-IgE antibodies and labelled allergens did not lead to a satisfactory sensitivity.
**Conclusion:** There seems to be an overall good correlation between the positive results of the two methods which uses the classical allergosorbent-design.

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### Fluorescent Multiplex Arrays – New Tools for the Study of Gene-Environment Interactions

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**Background:** Current studies of gene-environment interaction in allergy rely on methods for the assessment of allergic sensitization and allergen exposure that require separate testing for each allergen-specific antibody or allergen. We have applied fluorescent suspension array technology to allow the simultaneous detection of total and allergen-specific IgE in serum or for detection of multiple allergens in environmental samples in a single quantitative test.
**Method:** Fluorescent multiplex arrays were developed for eight indoor allergens (Der p 1, Der f 1, Mite Group 2, Fel d 1, Can f 1, Mus m 1, Rat n 1 and Bla g 2) using monoclonal antibodies covalently coupled to fluorescent microspheres. A 10-plex array for the detection of total IgE and IgE specific to Der p 1, Der p 2, Fel d 1, Can f 1, Bet v 1, Phl p 5, Bla g 1, Bla g 2 and Asp f 1 was created using monoclonal anti-IgE antibodies and purified allergens. The multiplex arrays were validated by comparing results between the multiplex methods and established enzyme immunoassays.
**Results:** There were highly significant correlations between results obtained by the multiplex methods and established enzyme immunoassays with $p<0.001$ ($n = >70$). In addition, the sensitivity, limit of detection, reproducibility, intra-assay coefficient of variance (CV), and inter-assay CV of the fluorescent multiplex array was shown to be equal to or better than the ELISA methods.
**Conclusion:** Our results suggest that fluorescent multiplex technology will facilitate epidemiological studies of exposure and allergic sensitization such as birth cohort studies, population surveys and studies of gene-environment interaction. The arrays can, in principle, be expanded to include other allergens, molds and allergen-specific antibodies.

## 157

### Characterization of naturally processed and presented T cell epitopes of Bet v 1, the major allergen from birch pollen

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**Background:** CD4+ T cells are key players in Type I allergies. They recognize allergen-derived peptides in the context of MHC class II molecules on the surface of antigen presenting cells. Up to now, allergenic T cell epitopes were identified employing synthetic overlapping peptides representing the entire amino acid sequence of an allergen. We were interested to identify naturally processed and presented (NPP) peptides of an allergen.
**Methods:** Monocyte-derived dendritic cells were generated from 4 birch pollen-allergic patients and pulsed with recombinant Bet v 1. MHC class II:peptide complexes were purified from the cell surface. Bound peptides were eluted and sequenced by mass spectrometry. In parallel, Bet v 1-specific T cell lines were established from PBMC of each individual and mapped for epitope recognition. Synthetic peptides based on NPP peptide sequences were used to stimulate Bet v 1-specific T cell clones with known epitope specificity.
**Results:** In total, 27 NPP Bet v 1-derived peptides were identified. The mean peptide length was 18 amino acid residues, ranging from 14 to 28. All NPP peptides isolated from each patient correlated with the peptides inducing proliferation in Bet v 1-specific T cell lines. Furthermore, synthetic peptides representing NPP peptide sequences potently activated Bet v 1-specific T cell clones.
**Conclusion:** For the first time, naturally processed and presented peptide sequences of a relevant allergen were identified. These peptides match epitopes defined in allergen-specific T cell lines and activate allergen-specific T cell clones, which confirms their in vivo relevance.

# Abstracts

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### Bet v 1 concentrations in ambient air do not correlate with birch pollen counts

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**Background:** Exposure to allergens like Bet v 1, the major birch pollen allergen, is generally deduced from birch pollen counts in ambient air. Proof is lacking that pollen counts reflect exposure to this allergen. We already published that birch pollen collected directly from catkins varied 6-fold in Bet v 1 release between 2002 and 2003 (Buters et al., Int Arch Allergy Immunol 145:122-130, 2008, epub Sept 2007). We now determined Bet v 1 concentrations in ambient air.
**Methods:** At the hospital campus Biederstein in Munich, Germany, 562 m above sea level, 1.8 m above ground, we monitored simultaneously birch pollen counts with a standard Burkard pollen trap, and Bet v 1 in ambient air with a Chemvol® High-Volume sampler (900 l/min) with various cut-off points (>$PM_{10}$, $PM_{10\text{-}2.5}$, $PM_{2.5\text{-}0.12}$). Bet v 1 was extracted from polyurethane filters with 0.1 M $NH_4HCO_3$, pH8.0 and quantitated with a Bet v 1 specific ELISA.
**Results:** Peak birch pollen count in 2004, 2005, and 2006 were 729, 3522 and 1681 pollen/$m^3$/24h, respectively. At the same time peak Bet v 1 concentration was 148, 364 and 1390 pg Bet v 1/$m^3$, respectively. According to pollen count, 2005 showed the strongest exposure to allergens. In 2006, when pollen counts were half of 2005, Bet v 1 concentration in ambient air was about 4-fold higher than 2005, indicating that the content of Bet v 1 per pollen was 8-times higher than in 2005. Also, later in the season when pollen counts were low, Bet v 1 persisted at high concentrations in ambient air.
**Conclusion:** Seasons with low birch pollen emissions seem to be associated with high Bet v 1 concentrations in ambient air and vice versa. Especially at the end of the season high Bet v 1 concentrations are detectable, although pollen counts are low. Thus birch pollen counts do not necessarily reflect birch allergen exposure.

## 159

### Der p1; a major allergen from Dermatophagoides pteronyssinus, but is there a biological function for Der p1?

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House dust mite allergy is one of the most wide spread allergic diseases. Patients allergic to house dust mite react to Der p1 from *Dermatophagoides pteronyssinus*, which is proposed to be a cysteine protease. Furthermore, allergens from house dust mite cause high serum levels of IgE antibodies which are responsible for allergic diseases like allergic asthma, allergic rhinitis and atopic dermatitis. Mechanisms by which dust mite proteases might promote an allergic responses have been described extensively, but still remain largely unknown. Also, the biological function of Der p1 from *D. pteronyssinus*, a presumed protease mainly found in excrement of the house dust mite, has not been described yet. However, our *D. pteronyssinus* mites, which have been kept in culture for at least 5 years, remain constant despite the possibility of biological selection. Whole culture, mite bodies, excrement and partial purified Der p1 from *D. pteronyssinus*, extracts, (all produced by Citeq biologics) were tested for serine and cysteine and chitine protease activity. ELISA experiments have shown that the highest concentration of Der p1 is found in excrement of *D. pteronyssinus.* Excrement from *D. pteronyssinus* has a higher Der p1 activity than Der p1 from freeze dried extracts or partial purified Der p1 from whole culture, but does not have cysteine protease activity, only. The presence of large concentrations of endotoxins in the natural environment of the house dust mite could explain de presence of large concentration of Der p1 in excrement of *D. pteronyssinus.* However, chitin a major product from the outer part of the house dust mite and from the cell walls of fungi could also contribute to high levels of Der p1 in excrement from *D. pteronyssinus.* A high Der p1 content and high protease activity found in excrement from *D. pteronyssinus* is an intriguing question and makes it an ideal system to find out, biochemically, a possible mechanism for allergic sensitization.

## 160

### Relevance of mite sensitization in workers exposed to grain dust

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Apart from the house dust mites (HDM), storage mites belonging to the Acaridae family and Glycyphagidae family can cause allergic symptoms. Since storage mites are detected in rural environment mainly in relation with grain and foodstuff storage, in workers who are exposed to grain dust a higher risk of storage mite sensitization were discussed. The aim of this study was evaluate the prevalence of HDM and storage mite sensitization in grain dust exposed workers and in a reference group matched for gender, age and atopy. In addition, the concentrations of storage mite antigens were measured in workplace-related grain dust and in house dust samples. Sera of 64 male grain storage depot workers were tested for specific IgE (sIgE) to sx1 (inhalative atopy screening test), HDM- (*Dermatophagoides pteronyssinus*, Dp) and storage mites sensitization (Acarus siro, As; Lepidoglyphus destructor, Ld; Tyrophagus putrescentiae Tp) and the results were compared with 64 males without occupational grain dust exposure. Using specific sandwich-ELISAs the antigen content of As, Ld and Tp were determined in dust samples. 17 of 64 grain workers' sera (27%) were positive in the sx1-test and 15 sera (22%) had sIgE to at least one mite. 11 sera were positive to Dp, 4 of them exclusively to Dp and 7 additionally to storage mites. Four sera were exclusively positive to one or more storage mites. 11 sera of the reference group (17%) had sIgE to at least one mite, whereas two of them showed isolated IgE-responses to the storage mites. No significantly different sensitization pattern to storage mite existed between grain workers and the reference group. In all dust samples storage mite antigens were detectable, whereas in the workplace grain dust samples the mite antigen concentration were higher compared to the house dust samples. A considerable difference was found for As (9 µg/g in grain dust and 0.1 µg/g in house dust). Our results demonstrated that workers exposed to grain dust had no higher prevalence of storage mite sensitization compared to a matched reference group, even though higher concentrations of storage mite were detected in dust samples from workplaces compared house dust samples.

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### Decelerated Differentiation and altered functional properties of cord blood $CD34^+$-derived Dendritic cells in newborns at risk for atopy

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The atopic march frequently starts with an onset of atopic dermatitis in infants followed by allergic rhinitis and allergic asthma. IgE sensitization to common aeroallergens plays a central role in the pathophysiology of atopic diseases.

Therein, dendritic cells (DC), T cells and B cells play a key role. Since early prevention is regarded as an important corner stone in the management of atopic diseases, the identification of reliable markers detecting individuals at risk are of major interest. Thus, we investigated whether DC, T cells and B cells enriched from the cord blood of newborns at risk (AT) phenotypically and functionally differ from cells of newborns without any risk for atopy (NAT).
For this reason cord blood was collected from elective cesarian sections. Newborns were defined as „at risk", if at least one parent suffered from one atopic disease(s) and „at no risk", if parents showed no atopic disorders. $CD34^+$ stem cells and T cells were isolated from cord blood. $CD34^+$ were cultured to generate DC and then functionally characterized. Furthermore DC were stimulated with antigens and cocultured with autologous T cells to analyse stimulatory capacity and Th1/Th2/Th3 cytokine production. TCR spectratyping and functional assays with B cells have been performed by flow-cytometry and real time PCR.
$CD34^+$ from AT displayed delayed differentiation into DC with lower CD1a and CD207 expression. Furtheron, DC from AT showed lower density of MHC class II molecules and CD83, but higher expression of FcεRI. Moreover, DC from AT presented lower stimulatory capacity towards autologous T cells as well as differences in their capacity to take up antigens. T cells from AT produced higher amounts of Th2 and lower amounts of Th1 cytokines. T cell receptor spectratyping revealed a different TCR beta chain repertoire of T cells of high- and low risk infants as well as distinct B cell functions.
In view of these data we conclude that functional differences on the level of DC differentation, TCR beta chain repertoire as well as B cell function might serve as a diagnostic tool to identify children at risk and to initiate prophylactic strategies to prevent atopic diseases.

## 162

**Interleukin-10-differentiated human dendritic cells tolerize Th2 responses of atopic subjects by inducing the differentiation of regulatory T cells**

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We recently found that IL-10-treated dendritic cells can be used to treat 'asthmatic' mice, wherein they abrogate airway hyperresponsiveness and can dramatically reduce airway eosinophilia and Th2 cytokine expression, as well as serum IgE/IgG1. These effects are allergen-specific, critically dependent of the dendritic cells' expression of IL-10, and are associated with induction of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg). Herein we investigated the ability of analogous IL-10-treated human dendritic cells to similarly tolerize Th2 responses of atopic subjects T cells and to induce Treg differentiation. Human dendritic cells were differentiated from $CD14^+$ peripheral blood monocytes in the presence ($DC_{IL-10}$) or absence ($DC_{TNF}$) of IL-10, then pulsed with the subjects' specific allergen. We found that IL-10 inhibited the dendritic cells' maturation, as determined by their reduced expression of antigen-presenting molecules (CD40, CD80, CD86, HLA-DR) and stimulatory cytokines (IL-6, IL-12), but it augmented their expression of IL-10 and CD85j/ILT2. The allergen-presenting $DC_{TNF}$ ($^{all}DC_{TNF}$) were strongly stimulatory for MACS-purified autologous $CD4^+$ T cells, and the addition of $^{all}DC_{IL-10}$ to these cultures dose-dependently antagonized proliferation and Th2 cytokine (IL-4, -5, -13) expression by the $^{all}DC_{TNF}$-stimulated T cells. We found that a population of $LAG3^+CTLA4^+$, IL-10-secreting, $CD4^+CD25^+Foxp3^+$ T cells arose in longer-term co-cultures of allergen-presenting $DC_{IL-10}$ and $CD4^+$ T cells, but not when $^{all}DC_{TNF}$ alone were cultured with the T cells. When MACS-purified from these co-cultures, the $^{all}DC_{IL-10}$-induced Treg effectively suppressed the proliferation and Th2 cytokine production by autologous $^{all}DC_{TNF}$-activated T cells, in a contact-dependent manner. In conclusion, our data indicates that IL-10-treated human dendritic cells can tolerize the allergic response by driving the differentiation of regulatory T cells, in a manner analogous to that which occurs in $^{all}DC_{IL-10}$-treated asthmatic mice.

## 163

**Grass tablet sublingual immunotherapy (SLIT) for the treatment of allergic rhinoconjunctivitis**

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**Introduction:** A fast-dissolving, once-daily grass immunotherapy tablet (GRAZAX®, Phleum pratense, ALK-Abelló A/S) for treatment of grass pollen allergy has been developed, and the safety and efficacy results after 2 years of continuous GRAZAX® immunotherapy are reported
**Method and Materials:** A 5 year, randomised, parallel group, double-blind, placebo-controlled, multi-centre trial is on-going. The participants received double-blind treatment with sublingual immunotherapy grass tablet from autumn 2004 and until the end of the grass pollen season 2007 followed by 2 years of follow-up.
The participants are males and females, 18-65 years of age. They have a clinical history of moderate to severe grass pollen induced allergic rhinoconjunctivitis of 2 years or more requiring treatment during the grass pollen season. All participants had a positive skin prick test response (wheal diameter ≥ 3 mm) to Phleum pratense and a positive specific IgE to Phleum pratense (≥ IgE Class 2).
The symptom score was based on 6 symptoms (runny nose, blocked nose, sneezing, itchy nose, gritty feeling/red/itchy eyes and watery eyes) scored on a daily basis on a scale from 0-3. The medication score was based on use of Desloratadine (5 mg), Olopatadine eye drops (1.0 mg/ml), Budesonide nasal spray (32 µg/puff) and Prednisone tablets (5 mg/tablet).
**Results:** Participants treated with GRAZAX® scored statistically significantly lower than participants treated with placebo during the entire grass pollen season 2005 and 2006 ($p<0.0001$). The treatment effects during the second year were numerically higher than those observed during year 1 although they did not achieve statistical significance compared with year 1, possibly because the trial was not originally powered to detect differences between treatment years. The clinical changes were supported by a progressive effect on immunological parameters, including IgE, IgG4 and IgE-blocking antibodies.
Treatment with GRAZAX® was well tolerated with significantly fewer related adverse events in the second year of treatment. The withdrawal rate due to adverse events was below 1% overall.
**Conclusion:** Treatment with grass allergen tablets resulted in a 36% decrease in rhinoconjunctivitis symptoms. An additional 46% reduction in the use of symptomatic medication relative to
placebo was demonstrated. These results suggest a sustained benefit of grass tablet immunotherapy treatment over more than one season.

## 164

**Safety and Efficacy of Oral Immunotherapy with a Microencapsulated Ragweed Pollen Extract (MRPE) in Patients with Ragweed-induced Seasonal Allergic Rhinitis (SAR)**

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We present here our data with MRPE (Curalogic A/S). Our initial safety study utilized quantitative intradermal endpoint titration to demonstrate that MRPE was compositionally similar and comparable in relative potency (RP) to a standardized reference extract.
Six patients (pts) [mean age: 40; (range: 30-57); mn RW puncture skin test sensitivity (ΣE): 80mm (med: 82.5; range: 45-100)] had valid assays [correlation of coefficient: ≥0.92; slope: ≥13mm]. CD (standard deviation of the mn $\log_3$ RP): 0.595 [CDER upper limit: 0.68] demonstrated Curalogic RWE was not different from the comparator [RP: 85% (mn $\log_3$ RP: -0.4; SD: 0.595); thus the 2 RWE can be considered equal in potency [reference %: 71-141]. The Curalogic and comparator RWE potencies were 127 & 171% of the non-dialyzed reference RWE.
From a separate Phase II clinical trial, we present detailed subset analysis of our findings with respect to effect of treatment (TX) duration and TX dose, on safety and efficacy. 607 RW allergic pts [≥ 2 year history; positive prick skin tests (wheal ≥5mm; erythema ≥15mm); positive CAP IgE assay (≥ 0.7kU/L)] were enrolled into a DBPL-controlled SAR study. Pts were randomized to: MRPE at 40 Amb a 1 units (U)/day; 40 Amb a 1 U/wk; or PL. Pts were dosed ≤24 wks with TX starting 8-12 wks prior to the RW season.

Data analysis showed that MRPE at 40 Amb a 1 U/day is efficacious [36% reduction in Total Symptom Score (TSS) over PL (MRPE: 7; PL: 11) when TX was initiated 10 wks prior to the RW pollen season]. The table summarizes the statistical results:

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| TX DURATION | # PATIENTS | TSS REDUCTION | P VALUE |
|---|---|---|---|
| < 8 Weeks | 252 | 4% | 0.6 |
| > 8 Weeks | 270 | 12% | 0.07 |
| > 9 Weeks | 166 | 27% | 0.004 |
| >10 Weeks | 86 | 36% | 0.007 |
| >11 Weeks | 55 | 36% | 0.04 |

MRPE was well tolerated with mild to moderate gastrointestinal side effects experienced in only 3.3% of pts relative to PL.

The findings from these studies demonstrate that oral immunotherapy with a novel microencapsulated method of allergen delivery is both safe and efficacious when used in an appropriate TX dosing regimen. Additionally, the results of our current multicenter trial with MRPE (n=545), incorporating these clinical observations, will be reviewed.

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**Sublingual latex immunotherapy: safety and efficacy in Anaesthesiologist with latex professional exposure**

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**Background:** Natural rubber latex (NRL) is one of the main triggering causes of allergic disorders, i.e. asthma, urticaria, angioedema and anaphylaxis, in sensitized patients. Specific immunotherapy can help improving symptoms related to NRL allergy. **Objectives:** The aim of this study was to evaluate the safety and efficacy of sublingual immunotherapy (SLIT) with a commercially available ammoniated NRL extract in an anesthesiologist suffering with severe NRL allergy. **Methods:** A 42-year-old male healthcare worker was referred to our office as a possible case of sensitivity to rubber products. He was an anesthesiologist with a suggestive and progressively worsening history of immediate-type hypersensitivity after exposure to latex. He experienced the following allergic symptoms: itchy, red, watery eyes with sneezing and runny nose, coughing, wheezing, chest tightness and dyspnea after wearing latex gloves or just by exposure to the surgery room. Skin prick test to latex and the latex glove test were both positive. These tests were done before and 3, 6, 9 and 12 months after beginning SLIT. Oral allergy symptoms were also monitored. Test of exposure to latex in surgery room was done 4 weeks before and after 12 months of SLIT. Sublingual latex immunotherapy (SLIT latex; ALK-Abellò Italy) was performed according to the manufacturer's suggestion. **Results:** No side effect related to SLIT was observed. A significant improvement of symptom and medication scores as compared to baseline values was observed after 3 months ($p < 0.01$) with a further amelioration after 1 year of treatment ($p < 0.0005$). The symptom score with foods triggering oral allergy test also significantly decreased ($p < 0.05$) after SLIT. After 12 months the anesthesiologist had no more symptoms after exposure to NRL in surgery rooms. **Conclusion:** Latex SLIT was safe and effective in a NRL allergic healthcare professional with latex hypersensitivity .

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**Vaccination with a Modified Vaccinia Virus Ankara-based Vaccine prevents allergen-specific sensitization**

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**Background:** Currently no treatment is available for food allergy and strict avoidance of the allergenic food remains the only way to manage the allergy. New strategies leading to a safe and efficacious food allergy treatment are required. **Objective:** Modified vaccinia virus Ankara (MVA), which allows high levels of expression of recombinant protein in vivo and gives rise to a Th1-biased specific immune response, was used as a prophylactic vaccine in a murine model of ovalbumin (OVA) food allergy.
**Methods:** An MVA-OVA vector vaccine was prepared. Female BALB/c mice were vaccinated twice with MVA-OVA, followed by sensitization with OVA plus alum. OVA-specific IgE activity was measured by mediator release from rat basophilic leukemia cells, whereas specific IgG subclass titers were determined by ELISA.
**Results:** Expression of immunologically active OVA in mammalian cells was demonstrated. OVA-specific IgE levels in sera from MVA-OVA-vaccinated mice were reduced and appeared delayed. The vaccine-mediated immune modulation was dose-dependent; the highest vaccine dose protected 50% of the animal from allergic sensitization. Upon sensitization similar OVA-specific IgG1 titers were found in all mice, but the OVA-specific IgG2a antibody levels were strongly increased in MVA-OVA-vaccinated mice signifying a Th1-biased and, non-allergic immune response.
**Conclusion:** Prophylactic vaccination with MVA-OVA delays and in part even prevents the onset of a successful allergen-specific sensitization. Recombinant MVA, which fulfills the requirements for clinical application, is a promising candidate vector for the development of novel approaches to allergen-specific prophylactic vaccination and specific immunotherapy (SIT).

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**Mepolizumab, a humanised anti-IL-5 monoclonal antibody, as treatment of severe nasal polyposis.**

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**Background:** 90% of nasal polyps are characterised by prominent eosinophilia. IL-5 is the key driver of eosinophilic differentiation and survival. The objective of the study was to investigate the therapeutic potential of inhibiting IL-5 using a humanized monoclonal antibody as treatment of severe nasal polyps.
**Methods:** 30 patients with severe nasal polyposis (grades 3-4) refractory to corticosteroid therapy were randomized in a double blind fashion to receive either 2 single IV injections (28 days apart) of 750mg mepolizumab (n=20), or placebo (n=10). Changes in nasal polyp score and in comparative nasal polyp score were assessed relative to baseline (week 0) at 1 and 2 months post last dose (week 8 and 12). A 1-point reduction in these endoscopic assessments was considered clinically significant (Tuncer 2003, Lund 1998). CT scans were also performed at week 8.
**Results:** There were no differences in baseline values between the two groups. A significant reduction was observed in nasal polyp score with mepolizumab at week 8 (60% vs 10%, p=0.011) and week 12 (65% vs 20%, p=0.025). 65% and 70% of patients on mepolizumab showed a 'much better' or 'better' nasal polyp score compared to 10% and 20%on placebo at week 8 and at week 12, respectively. Over 50% of subjects on mepolizumab demonstrated improvement on blinded assessment of CT scans at week 8. The need for surgery was also reduced in the group on mepolizumab with 15% of patients requiring surgery compared with 50% on placebo at week 12. Individual response and magnitude of response were consistently maintained across the endpoints analysed.
**Conclusion:** Mepolizumab was safe and well tolerated in all patients and significantly reduced the size and volume of nasal polyps for at least to 2 months post dosing. IL-5 inhibition is a potential novel therapeutic approach as replacement for systemic steroids and to prevent/delay need for surgery in patients with severe nasal polyposis. (supported by GSK, EUtracCT No 2005-005113-11)

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**Lung function at 10 years is not improved by early corticosteroid treatment in asthmatic children.**

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**Background** Possible long term effects of inhaled corticosteroid (ICS) treatment for asthma on lung function development in childhood is debated. In view of lung function at birth, we aimed to assess if early use of ICS influenced lung function at ten years of age.

**Methods** A ten year follow-up study of 614/802 children (mean age 10.9±0.9 years) with lung function measurements at birth in the Environment and Childhood Asthma study in Oslo included information on ICS treatment (124 with history of asthma) obtained at two and 10 years by parental interviews. Main outcomes at 10 years were the best values (% predicted and z-scores) of forced expiratory volume in one second ($FEV_1$) and mid-expiratory flow ($FEF_{50}$). The main explanatory factors were never, past or current use of ICS and z-scores of the tidal flow-volume ratio $t_{PTEF}/t_E$ at birth.
**Results** ICS treatment, reported by 11.9 % of children in the population sample and 71.6 % with current asthma, did not significantly influence lung function at 10 years. The best values (and Z-scores) of $FEV_1$ and $FEF_{50}$ were similar ($p>0.1$) in subjects receiving ICS during and after 0-3 years of age, after three years only or currently compared to steroid naïve children. Almost half of the change in lung function 0-10 years was explained by gender, a history of asthma and $t_{PTEF}/t_E$ at birth.
**Conclusions** Inhaled corticosteroid treatment for asthma, reported in every eighth child by age 10 years, did not significantly improve lung function from birth to 10 years.

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### Expression of functionally active Fcε and Fcγ receptors on mouse superior cervical ganglion neurites

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Others have reported the presence of the high affinity FcεR1 and FcγR1 on dorsal root ganglion neurites (Neuroreport 2004, 15: 2029; FASEB J 2004, 18: 182). We wished to determine if α, β and γ subunits of FcεR1 were present on neurites and whether the DNP-specific IgE sensitized neurites themselves could be activated by specific antigen. Furthermore, we have compared these observations with neurites sensitized to DNP-specific IgG and examined the neurites for expression of various Fcγ receptors. Superior cervical ganglia (SCG) from 2-3 day old BALB/c mice were cultured in the presence of cytosine arabinoside. Calcium mobilization and activation of fluorphore (Fluo 3) was used as an index of cellular activation and assessed by confocal fluorescence microscopy. SCG were stimulated with different concentrations of antigen/DNP-HSA (0.1-1000 ng/ml) to study concentration-dependent neurite responses to IgE and IgG. The fluorescent increase in IgE- or IgG-sensitized neurites was dose-dependent. However, the IgG response was less pronounced, and higher concentrations of antigen were needed (500 ng/ml; 57% response) compared to IgE-sensitized neurites (10 ng/ml; 75% response). The neurite activation was antigen-specific as it did not occur with non-haptenated protein. In addition, single cell activation was studied by applying a spritzer close to the cell body or neurite making it possible to study the movement of the response from there to surrounding neurites. After puffing on the antigen (10 ng/ml), there was an instantaneous increase in fluorescence in the closest cell body or neurite preincubated with IgE. Subsequently, fast movement of the signal towards attached neurites was observed. In this system, no response was seen in the IgG-sensitized neurons at this concentration. The expression of the FcεR1 and its specific sub-units was confirmed by immunocytochemistry on the cultured neurites. Using reverse transcriptase (RT)-PCR the presence of mRNA for FcεR1 alpha, beta and gamma sub-units was detected as well as for the α sub-unit of FcγR, FcγIV and FcγRII or III (as the primers used did not distinguish between FcγRII and III). The biological significance of these findings will be discussed.

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### Activation of Sensory Nerves by a Prostaglandin D2 Metabolite

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Allergen exposure and other processes that lead to tissue mast cell activation, are often associated with symptoms of sensory C-fiber nerve activation. Depending on the tissue involved these symptoms include sneezing, coughing, itching, vasodilation, mucosal irritation, hypersecretion, etc. The mediators responsible for the activation of sensory nerves in allergic disease have not been fully worked out. Mast cell activation leads to PGD2 production, but the effect of PGD2 on visceral sensory C-fibers has received little attention. Recently a member of the transient receptor potential (TRP) family of ion channels, TRPA1, was found to be expressed by many C-fiber neurons in the somatosensory system. TRPA1, like other TRP channels, is a cation channel that when opened results in nerve activation. Using single cell RT-PCR we noted that 12 of 12 lung-specific sensory neurons that express the capsaicin receptor TRPV1 (a marker of C-fiber neurons) also expressed TRPA1. TRPA1 was expressed in only 1 of 12 TRPV1-negative neurons. Strong electrophilic substances such as mustard oil, cinnamaldyhde, and acrolein, gate TRPA1 through a covalent modification of cysteine residues on the N-terminal of the channel protein (*PNAS 103:19564-19568, 2006*). We reasoned that because the PGD2 metabolite 15-deoxy-$\Delta^{12,14}$-PGJ2 shares chemical features with these known activators of TRPA1, it may also gate the channel. We found that PGD2 itself failed to activate sensory C-fibers, but 15-deoxy-$\Delta^{12,14}$-PGJ2, at endogenously relevant concentrations (1-100 μM), stimulated 75% of capsaicin-sensitive visceral sensory neurons (n=71). The stimulation was similar in magnitude to that seen with capsaicin, and was inhibited by a TRPA1 antagonist. In addition, 15-deoxy-$\Delta^{12,14}$-PGJ2evoked large calcium responses, and inward electrical currents in HEK cells transfected with human TRPA1, but not in wild type HEK cells. These data support the hypothesis that PGD2, via it oxidative metabolite, may be a mast cell mediator that participates in allergen-induced visceral sensory C-fiber activation. Supported by NIH (USA).

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### Genetic communication between mamallian cells via exosomal shuttle of RNA (esRNA)

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Exosomes are 50-100 nm large vesicles that are released into the extra cellular environment by many different cells. Exosomes have been shown to contribute to antigen presentation (Raposo et al., 1996), and vaccines for cancer using exosomes are under clinical development. We have recently found substantial numbers of RNA in exosomes from a number of different cells, including those produced by mast cells (mouse and man), human macrophages, human monocytes, B-cells and dendritic cells. RNA-containing exosomes can also be detected in human plasma. The exosomal RNA seems to be highly selective, and is in many way distinct from the donor cellular RNA. Using microchip analysis, we have in mast cell exosomes identified approximately 1300 mRNA and 121 microRNA (size 19-21 nucleotides), many of which are unique and not present in the cytoplasmic RNA pool of the donor cell. A key finding in our studies is that mast cell exosomes have the capacity to donate their RNA to another mast cell, and subsequently affecting the protein production in the recipient cell. Thus, we have shown that exosomes derived from mast cell cultures can deliver their RNA to other mast cells. By adding exosomes from mouse cells to a culture of human cells, we identified newly produced mouse proteins in the human cells, which originated from the mouse exosomal mRNA. This shows, for the first time, that RNA can be transferred between mammalian cells by an extracellular exosome based transport mechanism, which may have vast implications on cell to cell communication, regulation and signalling. In effect, the complexity of communication between cells seems to be much more advanced than previously thought, as the gene expression in one cell now has been shown to affect the protein production in another cell, after transfer of mRNA between cells via exosomes. The exosomal shuttle of RNA (esRNA), can be seen as an endogenous form of natural "gene therapy", which putatively could be used to enhance gene therapy in disease.

Valadi, H. et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9(6):654-9.

## 172

### Inducing an Anergic State in Mast Cells and Basophils without Secretion: Theoretical and Practical Implications

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New inhibitors of syk and PI3K are both potent and relatively selective but

# Abstracts

because these kinases are central signaling components in the activation of many cells in the immune response, it is difficult to envision safe methods of using them chronically for the treatment of atopy. The following studies suggest one way they could be used acutely. IgE-mediated stimulation of mast cells and basophils results in events that parallel activation but serve to down-regulate the response (desensitization). Recent studies have shown that syk activity is not required to observe continued activity of several specific down-regulatory pathways (e.g., loss of syk by ubiquination). In contrast, secretion depends on the activity of both syk and PI3K. We tested whether stimulation with antigen, in the presence of inhibitor would ablate secretion while simultaneously allowing desensitization. The desensitized state was assessed after a relatively short period of time (45-60 min.) by removing the inhibitor and re-stimulating the cells. Peripheral blood basophils, cultured-derived human mast cells and in situ stimulated airway mast cells (in organ baths) were examined. Antigen caused 35 ±65% and 62 ±10% histamine release from basophils and mast cells, respectively; and it caused an 87±5% histamine/LTD4-dependent contraction of human isolated bronchi. All of these responses were blocked > 95% by the syk inhibitor. Re-challenging the preparations with antigen, after first washing out the drug and antigen, revealed that near complete desensitization (92-100%) occurred in each case. In control experiments we demonstrated that the syk inhibitor was readily reversible and easily washed out of the cells and tissues. Thus, even though the syk inhibitor nearly abolished the antigen-induced secretion from mast cells and basophils, it had little effect on the pathways involved in desensitization. A similar result was found when using the PI3K inhibitor, LY294002. These results suggest that it would be possible to administer the inhibitor in vivo and then introduce antigen without a significant induction of mast cell/basophil secretion. But importantly, after drug had been metabolized, the tissue mast cells and basophils should be unresponsive to stimulation with antigen that may still be present (e.g., rush immunotherapy).

### 173
**Allergen-S-layer fusion proteins: Promising candidate vaccines for specific immunotherapy of Type I allergies**

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Ideal vaccines for specific immunotherapy of Type I allergies should display a reduced risk to induce IgE-mediated side effects, stimulate the innate immune system of allergic individuals and amplify the modulation of the disease-eliciting Th2-dominated allergen-specific response towards a more physiological response. S-layer proteins form crystalline arrays on the cell surface of non-pathogenic Gram$^+$ bacteria. This self-assembly into molecular lattices may contribute to their recognition as danger signals by the human immune system. A recombinant self-assembling fusion protein of the bacterial S-layer protein of *Geobacillus stearothermophilus* ATCC 12980 and the major birch pollen allergen Bet v 1, rSbsC-Bet v 1, was generated. This allergen-S-layer fusion protein exhibited reduced allergenicity and induced IFN-γ and IL-10 synthesis in Bet v 1-specific Th2 clones. Moreover, rSbsC-Bet v 1 stimulated immature monocyte-derived dendritic cells (mdDC) expanded from birch pollen-allergic patients to mature and to synthesize IL-10 and IL-12. These mdDC promoted the differentiation of autologous naïve T cells into IFN-γ-producing cells in an IL-12-dependent manner. In parallel, a substantial number of naïve T cells developed into IL-10-producing CD25$^+$Foxp3$^+$CLTA-4$^+$ cells that actively suppressed anti-CD3-stimulated CD4$^+$CD25$^-$ T cells. Evaluation of possible differences between the self-assembled and monomeric forms of allergen-S-layer fusion proteins regarding their capacity to bind IgE and to induce mediator release as well as to stimulate Bet v 1-specific T cells revealed that both forms are hypoallergenic and activate Bet v 1-specific T cell clones with known epitope specificity.
In summary, rSbsC-Bet v 1 meets several desired requirements for an ideal allergy vaccine: less IgE-binding and reduced mediator-releasing capacity which may result in less IgE-mediated side effects; immune stimulatory effects on the innate immune system and immune modulatory effects on naïve and allergen-specific effector T cells indicating that rSbsC-Bet v 1 will simultaneously support immune deviation and the induction of regulatory T cells; finally, a constant ratio between allergen and adjuvant which should improve consistency of the product used for SIT. Therefore, allergens genetically fused to S-layer proteins are promising future vaccines for specific immunotherapy of atopic allergies.

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### 174
**An IgE-based Polyplex Allergen Gene Vaccine That Targets Dendritic Cells as an Novel Therapuetic Approach for Severe Food Allergy**

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Gene vaccination represents a very promising approach for treatment of severe food allergy as it has been shown to down regulate allergic responses and does so by only exposes the subject to the peptides produced *in vivo*. Indeed, gene vaccination holds great promise in other fields such as infectious diseases and cancer. However the inability to target and express the gene(s) of interest in antigen presenting cells (APCs) has been a major impediment. To overcome this obstacle, we have designed and expressed a genetically engineered human Fc (CHε2-4) plus poly-lysine (45 aa) fusion protein ("hEPL") that binds plasmid-DNA to form a polyplex as a novel form of gene vaccine. The Fcε portion of the protein targets the FcεRI on APCs while the poly-lysine portion binds and carries the plasmid-DNAs containing the gene of interest into the targeted cells. By placing the plasmid gene of interest under the control of the dendritic cell specific *fascin* promoter rather than a promiscuous promoter, the protein can be specifically expressed in dendritic cells. We have also designed and expressed a more sophisticated version of this hEPL that incorporates a HIV tat peptide sequence and the nuclear localization signal peptide to provide for enhanced membrane fusion and nuclear localization. Characterization of the affinity column purified mammalian cell expressed hEPL protein expressed i showed that it is assembled primarily as dimers, an important feature to achieve high affinity FcεRI binding. The hEPL was shown to be capable of binding plasmid DNA in a dose-dependent manner and of targeting FcεRI *in vitro*. hEPL polyplexes containing plasmids encoding the major peanut allergen Ara h1 were injected into human FcεRIα transgenic mice, mice that express humanized FcεRI on their APCs. Animals given injections of 10µg Arah1-DNA bound to 15µg of EPL at day 0, 7 and 14 demonstrated the induction of an Arah1 IgG2a response by day 28 which futher increased out to day 42. We are testing whether this novel gene vaccine can be used prevent or treat established IgE mediated food allergy or can be used as a vaccination stratagy against infections or cancer.

### 175
**GATA-3-specific DNAzyme - a novel approach toward asthma therapy**

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The transcription factor GATA-3 has been shown to regulate development and activity of Th2 lymphocytes and more recent investigations demonstrated that it is also involved in the activation of eosinophils, mast cells and NKT cells. Based on these data GATA-3 is considered the "master transcription factor" of Th2-driven inflammatory immune responses including allergies and therapeutic downregulation may therefore represent a promising approach toward a novel therapy of allergic diseases including allergic bronchial asthma. To address this approach we developed a GATA-3 mRNA-specific DNAzyme, a molecule that combines specificity of antisense molecules with an inherent RNA-cleaving enzymatic activity, and analyzed its efficacy in various experimental asthma models.
Therefore, a series of GATA-3-specific DNAzymes were generated and the most active DNAzyme was selected for subsequent use after in vitro testing. The anti-allergic capacity of this DNAzyme was investigated after intranasal application using ovalbumin (OVA)-based mouse models of allergic airway inflammation and directly compared to GATA-3-specific antisense-DNA and siRNAs.
Using a preventive treatment protocol, all GATA-3-specific antisense molecules were able to significantly reduce the allergic inflammatory response in the lung as shown by reduced airway goblet cell numbers and reduced tissue inflammation revealed by histopathological analysis. Lung reactivity to methacholine was significantly improved in comparison to the untreated group. With respect to eosinophil numbers in the bronchoalveolar lavage (BAL) and the influence on BAL IL-5/IFN-γ ratios the DNAzyme exerted most pronounced effects. Comparable effects were also observed in a therapeutic model where DNAzyme treatment started at a time point when the allergic inflammatory response was already established and in a chronic model where treatment was initiated after

several weeks with recurrent airway allergen challenges. Additionally, extensive in vitro and in vivo studies revealed no Toll-like receptor (TLR) 9-activating off-target effects of the GATA-3-specific DNAzyme.
In conclusion, therapeutic downregulation of GATA-3 has been validated to be a promising approach for the treatment of allergic inflammatory responses in the lung based on its central role in the regulation of Th2-driven immune responses. Intranasal application of a highly specific DNAzyme provides an interesting new tool to accomplish this goal.

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**News from the mimotopes: Therapeutic tools against allergy and cancer.**

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Antibodies recognize conformational epitopes which can be mimicked by small peptides drawn from phage display libraries. Being epitope mimics, they are termed mimotopes. During the recent years we have characterized B-cell epitopes of the most important allergens and tumour antigens by this technique, rendering visualization of the specific structural epitopes as well as the possibility of directed induction of epitope-specific immune responses through vaccinations with the mimotopes. The biological significance of IgG antibodies induced by tumour mimotope vaccines could be demonstrated in vitro and in vivo. The biological significance of mimotope-induced anti-tumor IgE antibodies was presented at the last CIA symposium, introducing the novel concept of allergooncology. However, the proof of concept for mimotopes in the allergy setting was until now missing. Here, we show for the first time the therapeutic effect of mimotopes in a murine model of asthma. Whereas sensitized, but non-treated animals exhibited bronchial eosinophilia and mucous secretion, mimotope treated mice showed an impressing remodelling and could be cured from disease. The effect was superior to treatments with the allergen itself, possibly due to a lack of T-cell epitopes within the mimotopes. The effect was equally pronounced with peptide mimotopes as well as with mimobodies, i.e. anti-idiotypic Fab fragments to allergen- specific IgE. Going one step further, the peptide mimotopes were translated into DNA minigenes. Interestingly, tumour as well as allergy minigenes were capable of inducing specific IgG antibodies, pointing towards a further novel treatment option, whenever epitope-specificity is an important prerequisite. In both cases, the induction of detrimental specificities like growth-stimulating or anaphylactogenic antibodies can definitely be ruled out.

The work was supported by the Austrian Science Fund FWF, grants SFB-F01808-8 and P18238-B13.

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**Anti-IgE in severe atopic eczema with high serum IgE levels**

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**Background**: Anti-IgE has been used successfully in allergic bronchial asthma. Here we report preliminary experience with low dose anti IgE therapy in patients with severe atopic eczema (AE) and high serum levels of total IgE.
**Patients and Methods**: A total of 11 patients (4 female, age 24 – 47) with high serum IgE levels (2900 - 39000 kU/l) and a history of allergic rhinoconjunctivitis and allergic bronchial asthma were treated with anti-IgE (omalizumab) with a fixed schedule of 10 cycles of 150 mg s.c. in two week intervals. All patients had undergone several standard eczema therapies (Cyclosporin A, UV, systemic steroids) before enrollment and were free of systemic treatment for a period of 6 weeks prior to study inclusion.
**Results**: 6 of the 11 patients showed remarkable improvement in skin symptoms (SCORAD reduction over 25%), 3 patients showed minimal changes and 2 patients deteriorated. In peripheral blood the ratio of IgE/IgG-specific transcripts decreased markedly and mostly in patients with good clinical response, while the IgE/IgG-mRNA ratio increased in 2 patients without response or with deterioration. In the serum, total IgE (bound and free IgE) showed a slight increase during therapy while free IgE was basically unchanged. IgM and IgG levels also remained unaltered. In patients with clinical improvement also serum levels of the chemokine TARC/CCL17 showed a marked reduction.
**Conclusions**: Low dose of omalizumab ameliorated skin symptoms in a selected number of adult patients with severe atopic eczema and high serum IgE. The clinical improvement was paralleled by a reduction in IgE/IgGmRNA ratio and serum TARC levels. It is concluded that further studies with omalizumab in patients with severe AE are warranted and might yield new information on pathophysiology and regulation of IgE in atopic eczema.

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