CIA

26TH SYMPOSIUM - MALTA

5TH - 10TH MAY 2006

Cellular & Molecular Targets in Allergy & Clinical Immunology

5 - 10 May 2006 ~ Malta

COLLEGIUM INTERNATIONALE ALLERGOLOGICUM

CIA

Letter of Invitation

Dear Colleagues:

On behalf of the Collegium Internationale Allergologium, it is our great pleasure and honor to welcome you to the 26th Symposium in Malta. We believe that the program presented over the next week will be both scientifically and socially rewarding.

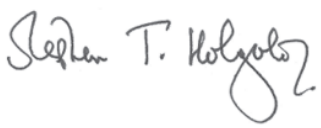
This Symposium, with over 300 registered attendees and nearly 200 oral and poster presentations, is already a huge success and will surely be remembered by members of CIA for years to come.

With the enormous developments that have occurred in translating molecular science into diagnostic and therapeutic applications, we have decided to focus the 2006 meeting on *"Cellular & Molecular Targets in Allergy & Clinical Immunology"*. As in previous years, the majority of the scientific program will be given over to free communications that will be given either as oral presentations or as poster discussions.

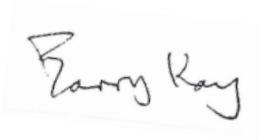
We are also excited and pleased to offer attendees and guests a great social program and Maltese hospitality at its best. Guided tours are available for attendees and guests during each day of the meeting. We invite you to discover the Maltese islands, known for their friendly people, year-round sunshine and intriguing history.

Thank you again for attending the 26th Symposium of the Collegium Internationale Allergologium. From the beginning, the Collegium was intended to be an exclusive club of friends sharing the common pursuit of excellence in research in a spirit of intellectual exchange, and we will strive to fulfill this vision over the next week.

Yours sincerely,



Stephen T. Holgate
Symposium Organizer



Barry Kay
Symposium Organizer

Johannes Ring
President

Gianni Marone
Vice-President

General Information

Venue

The 26th Symposium is held on the beautiful island of Malta. Its 4,000 year-old history is still alive in its mystical, prehistoric temples, impressive archeological sites, monumental palaces and churches now married with modern and advanced living. Malta's Mediterranean and Maltese cultural identity stems from those who have conquered it in the past, including the Romans, the Arabs and the Knights. The stunning beaches and the clear Mediterranean Sea are the venue for relaxation and enjoyment.
The symposium scientific program will take place at the Westin Dragonara Resort Conference Center, St. Julians, situated just five minutes away from the island's major highway which connects all major venues and local attractions.

Westin Dragonara Resort

Dragonara Road

St. Julian's

STJ02

Malta-Europe

Tel. +356 21 381000

Fax. +356 21 381347

Coffee Breaks and Lunches

Coffee breaks and lunches are included in the registration fee and will be served daily. Coffee will be served in the North Foyer of the Conference Center and lunch will be in the Westin Dragonara Resort's Terrace Restaurant; please check the *Program at a Glance* for exact times.

Currency

The unit of currency in Malta is the Lira (Lm), which is divided into 100 cents. Banks, ATMs and exchange bureaux can be found all over the islands. Banks are generally open between 8:30 a.m. and 12:30 p.m. Monday to Friday, and until 11:30 a.m. on Saturday. The Exchange Bureaux at the international airport is open 24 hours a day. Many hotels, shops and restaurants accept foreign currency. Most hotels and restaurants, as well as many shops, accept Access, American Express, Carte Blanche, Diners Club International, MasterCard and Visa. One Maltese lira (Lm1) is about US $2.50 and € 2.30.

Electricity

Electricity supply is 240 volts, single phase, 50 cycle. The square-fitting standard three-pin British plugs and sockets are used.

Internet Café

The Internet Café will be located in the Westin Dragonara Resort Conference Center, Roman Room; access is free to all attendees and guests.

Language

The official language of the CIA Symposium is English, and the official languages of Malta are Maltese and English. Almost all the Maltese speak English. Maltese is a Semitic language written in the Roman script comprising a vast element of words of Latin origin. Italian is also widely spoken.

Name Badges

Your personal badge is your entrance to sessions and social events, these can be picked up at the registration desk, located in the Cloak Room of the Westin Dragonara Resort Conference Center.

Proceedings

The 26th Symposium presentations are planned to be published as a supplement to the *Allergy & Clinical Immunology International – Journal of the World Allergy Organization (ACII-JWAO)* (Hogrefe and Huber Publishers). Manuscripts to be submitted online; please visit **www.ciaweb.org** for detailed guidelines.

Registration

The Registration desk is located in the Cloak Room of the Westin Dragonara Resort Conference Center.

Hours:

Friday, 5 May 2006: 14:00 - 20:00
Saturday, 6 May 2006: 7:30 - 13:00
Sunday, 7 May 2006: 7:30 - 13:00
Monday, 8 May 2006: 7:30 - 11:30
Tuesday, 9 May 2006: 7:30 - 13:00
Wednesday, 10 May 2006: 7:30 – 12:15

Registration Fees

Registration Fees for Attendees includes:

Transportation to and from the airport
Daily coffee breaks
Daily lunch
Admission into all oral and poster sessions
Social Events

Registration Fees for Guests includes:

Transportation to and from the airport
Daily lunch
Social Events

Speaker Ready Room

There is a Speaker Ready Room in the Westin Dragonara Resort Conference Center, Arabian Room. Speakers will be able to check and register their presentations before the sessions.

Hours:

Saturday, 6 May 2006: 7:30 – 13:00
Sunday, 7 May 2006: 7:30 – 13:00
Monday, 8 May 2006: 7:30 – 11:30
Tuesday, 9 May 2006: 7:30 – 13:00
Wednesday, 10 May 2006: 7:30 – 12:15

Time Zone

Malta is on Central European Time (CET), which is one hour ahead of Greenwich Mean Time (GMT). The islands are six hours ahead of Eastern Standard Time (EST) and nine hours ahead of Pacific Standard Time (PST).

Tipping

A gratuity of 15% is expected in hotels and 10% in restaurants, if a service charge is not included in the bill. Most services are tipped about 10%.

General Information

Transportation

Airport shuttles will be arranged for delegates upon arrival and departure.

Wine & Cheese Poster Sessions

Poster presentations are separated by category into a number of Wine & Cheese Poster Sessions. A moderator will act as your 'tour guide' and spend about 1 hour on the session topic. Each topic will be briefly introduced and each poster presenter will give a 3 minute explanation of their poster and answer questions. Each Wine & Cheese Poster Session is 2 hours long, allowing you to 'tour' the 2 chosen categories of the night for an hour each. Please see the *Program at a Glance* for exact times. All poster sessions will take place in the Phoenician, Castillian and Carthaginian rooms.

Social Events

Welcome Reception

A Welcome Reception will be held on Friday, 5 May 2006, from 19:00 – 21:30 at the Westin Dragonara Resort Reef Club. The Reef Club is set on the water's edge, by the turquoise waters of the pool. Open bar and an assortment of appetizers will be served.

Maltese Dinner at the Villa Bologna

On the evening of Saturday, 6 May 2006, from 20:00 – 23:00, dinner will be provided at the Villa Bologna. Hidden behind high walls, Villa Bologna has been handed down in the same family since the 18th Century. Visitors enjoy the baroque statuary, fountains, ornamental trees and shrubs of this historical private garden. Formerly the residence of Lord Strickland, who was prime minister of Malta from 1927 to 1930, Villa Bologna was first constructed in 1745. Lord Strickland was a prominent figure in Maltese and British politics and his wife, Lady Strickland, oversaw the expansion of the villa and the gardens. Together with her friend Count Giuseppe Teuma Castelletti, they designed the Dolphin pond, Sunken pond, cactus garden and vine-covered pergolas that lead through the citrus groves of orange, lemon, grapefruit, and tangerine trees.

Dine Around – Free Night

Enjoy Maltese culture and cuisine on the night of Sunday, 7 May 2006, at your leisure. A listing of recommended restaurants is included in your conference pack with contact details, address, type of cuisine and the average cost of a 3-course dinner excluding drinks. For entertainment, choose from all that Malta has to offer, including classical, jazz and folk music; the latest blockbuster releases or art house films; theater and opera; and dance and baroque festivals.

Turkish Boat Ride (swimming included)

On Monday, 8 May 2006, symposium participants will board boats at Sliema and be taken to Comino to drop anchor near the Blue Lagoon, where a buffet style lunch will be served. The small island of Comino has been seen throughout history as an unsafe place to live. In 1416 the Maltese petitioned the Aragonese king, Alphonse V, to build a tower as a deterrent to the corsairs who made it their base. Construction on the tower did not begin, however, for 200 more years. After it was complete, people were still wary of making the island their home. Today, this feeling persists, only a handful of resident families and a single hotel occupy this forsaken but beautiful island. The journey back will take participants through the Grand Harbour, which offers an impressive spectacle not easily equaled by the harbors along the Mediterranean seashore. Visitors will be amazed by the grandeur and majesty of Malta's main port, with its imposing centuries-old fortifications and the historic Three Cities on one side and the Baroque city of Valletta on the other.

Gala Dinner at La Sacra Infermeria

An elegant dinner will be served on Tuesday, 9 May 2006, from 19:15 – 23:00, at one of the most commanding buildings in Valletta, the former *Sacra Infermeria*, built by the Order of St. John of Jerusalem in the 16th century. The Knights set out to create a city built as a fortress that also provided a luxurious place to live. The architectural style and grandeur of the historical building amazes all visitors. The halls of *La Sacra Infermeria* were once lined with comfortable beds for the sick and injured who were treated with the most advanced techniques and fed on silver platters coinciding with their social status.

Beneath *La Sacra Infermeria* there is a permanent exhibition dedicated to the Order of the Knights of St. John, next to the historic Fort St. Elmo. Guests catch a glimpse into the chivalrous world of the Hospitaller Knights by experiencing the sights and sounds of the period. Learn about the Crusades, The Great Siege of 1565, the building of the glorious hospital, the development of surgery, the origins of modern day healthcare and medicine and the ravages of plague. The attraction combines a mixed range of media including displays with full-scale figures and others with smaller scale dioramas interspersed with 30 centimeter high figures. The whole attraction houses over 100 figures, some in period costume.

La Sacra Infermeria was fully restored in 1979 and converted into what is now popularly known as the Mediterranean Conference Center. The building includes a total of nine halls and over 7,000 square meters. Presently, it is the largest conference center in Malta, situated in the capital city of Valleta, overlooking the breathtaking Grand Harbour on the eastern side of the city.

Optional Tours

All tours will depart at 9:00 from the Westin Dragonara Resort, with the exception of the tour of the Hypogeum and Tarxien Temples, which will depart at 8:00. The tours are all half-day tours and will return to the Westin Dragonara Resort in time for the included lunch, excluding the full day tour of Gozo.

Valletta and Malta Experience

Saturday, 6 May 2006 – 9:00

Participants will be guided through the bustling streets of the capital city of Malta, Valletta, on this half-day tour. The guide will point out some entertaining facts about several of the monumental buildings. The first point of interest will be the charming park, Barracca Gardens, which offers the famous panorama of the Grand Harbour. The journey continues to the St. John's Co-Cathedral, which contains the must-see Baroque riches that date back to the times of the Knights of Jerusalem, Rhodes and Malta. Afterwards, participants will visit the Grand Master's Palace for a view of the Staterooms or the Armory. Lastly, the tour will stop at the Malta Experience, a multivision show that depicts Malta's 7,000 years of history throughout the ages.

Price Per Person: $42.00 USD

This price includes transportation and entrance to St. John's Co-Cathedral, the Grandmasters Palace and the Malta Experience.

Gozo

Sunday, 7 May 2006 – 9:00

The full day tour of Gozo, Malta's sister island, begins with a

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General Information

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twenty minute ferry crossing. Malta and Gozo have a similar history and development; however, Gozo's countryside is greener and more spectacular, with flat-topped hills characterizing the landscape and coasts with rugged cliffs, penetrated by steep valleys and beautiful bays. Participants will then visit the temples of Ggantija, known as the most imposing and best preserved of the megalithic structures erected during the Copper Age (3600-2500 BC). It is said that these megalithic temples of the Maltese Islands are the most impressive monuments of European prehistory.
Also of interest in Gozo are the bays of Xlendi and Dwejra. Xlendi is a peaceful landlocked bay with superb blue green waters that has recently become a tourist destination. Dweejra Bay is a place of natural beauty on the western coastline of Gozo, which attracts many visitors due to its serene surroundings. Of particular interest is the Azure Window, the Inland Sea - a secluded bay surrounded by high cliffs.
A typical farmer's lunch will be served at Ricardo's in the citadel in Victoria. Free time will be given to browse area stores for Gozo's renowned woollen goods and handmade lace.

Price Per Person: $70.00 USD
The price includes transportation to and from the ferry from the hotel, transportation in Gozo, the Ggantija temples, the ferry and lunch.

Mdina, Dingli and Blue Grotto

Sunday, 7 May 2006 – 9:00
This half day tour takes participants to the central part of Malta, which is dominated by the ancient citadel of Malta. By traveling through the narrow and winding street of Mdina, the 'Silent City', it is easy to admire the large homes of the aristocracy. The group will also see the cathedral and the imposing bastions, which will give a great view over much of the island, and then continue to the Dingli Cliffs, the highest point of the island, where there will be an opportunity to view the grottoes from close by in "luzzu", the Maltese fishing boats.

Price Per Person: $35.00 USD
This price includes transportation and admission into the Luzzu at Blue Grotto; admission into Mdina Cathedral will depend on mass schedule.

Hypogeum and Tarxien Temples

Sunday, 7 May 2006 – 8:00

Tuesday, 9 May 2006 – 8:00
Those interested in Malta's early history will enjoy this half-day tour. The subterranean Hypogeum and nearby Tarxien temples will be visited. It is thought that the Hypogeum temple was first in use around 3600 BC when its natural cavities were used as a repository for the bones of the dead. As the cavities filled up, new chambers were cut deeper into the rock. An underground area of more than 500 square meters devoted to worship and burial and the bones of over 7,000 people have been found. The system of caves, passages and cubicles cut into the stone is similar to the interiors of megalithic temples.
The first Tarxien Temples dates to around 3100 BC and is the most elaborately decorated of the Maltese Temples. Unlike other Maltese Temples, Tarxien has three pairs of apses instead of the usual two. The Tarxien Temples have been called the most beautiful prehistoric remains in Europe.

Price Per Person: $48.00 USD
This price includes transportation and entrance into the Hypogeum. This excursion is limited to 15 participants per day.

Temples and Marsaxlokk

Monday, 8 May 2006 – 9:00
The tour includes an excursion to the south of the island, where the group will visit the Hagar Qim and Mnajdra temples. Hagar Qim, discovered under rubble in 1839, dates from around 2400 – 2000 BC. The largest megalith at Hagar Qim is some seven meters high and weighs around 20 tons. Many items of interest have been unearthed at Hagar Qim, and the site itself has connotations with a fertility cult. Other temple ruins stand a few meters away from the main temple. The Mnajdra Temple group stands isolated, about 600 meters further down cliff top. Mnajdra is made up of two sizeable temples and is thought to date from around 3400 BC. The masonry shows intricate knowledge of building techniques and excellent workmanship.
The second part of the tour will take place in the main fishing village of Marsaxlokk, located on the south eastern part of Marsaxlokk is the foremost fishing village and, perhaps, the most picturesque seaside locality in Malta. Fishing nets are often spread on the harbor to dry in the sun and, quite often, sturdy fishermen can be seen mending these nets. These activities, together with the modest houses by the seaside, give the area a charming and serene quality.

Price Per Person: $33.00 USD
This price includes transportation and entrance to the Hagar Qim and Mnajdra temples.

Noble Houses

Tuesday, 9 May 2006 – 9:00
The half day tour of the famous noble houses of Malta begins at the Casa Rocca Riccola in Valletta, home of Marquis Nicholas and Marchesa Frances de Piro. This 50 room home is still privately owned, and provides a unique historical perspective of Malta over the last 400 years through the collection of furniture, silver and paintings. In addition, a Museum of Costume and a World War II Air Raid Shelters exhibit have opened on the property over the last few years, providing a dramatic and exciting addition.
The second stop on this tour takes place in Naxxar, in the center of the island, where visitors will experience Palazzo Parisio. This magnificent palace and its beautiful surrounding garden has been in the Scicluna family since the middle of the 19th Century, when it was acquired from the Parisio family by the Marquir Giuseppe Scicluna. It was originally built in 1733 by the Portugese Grandmaster Manoel de Vilhena.

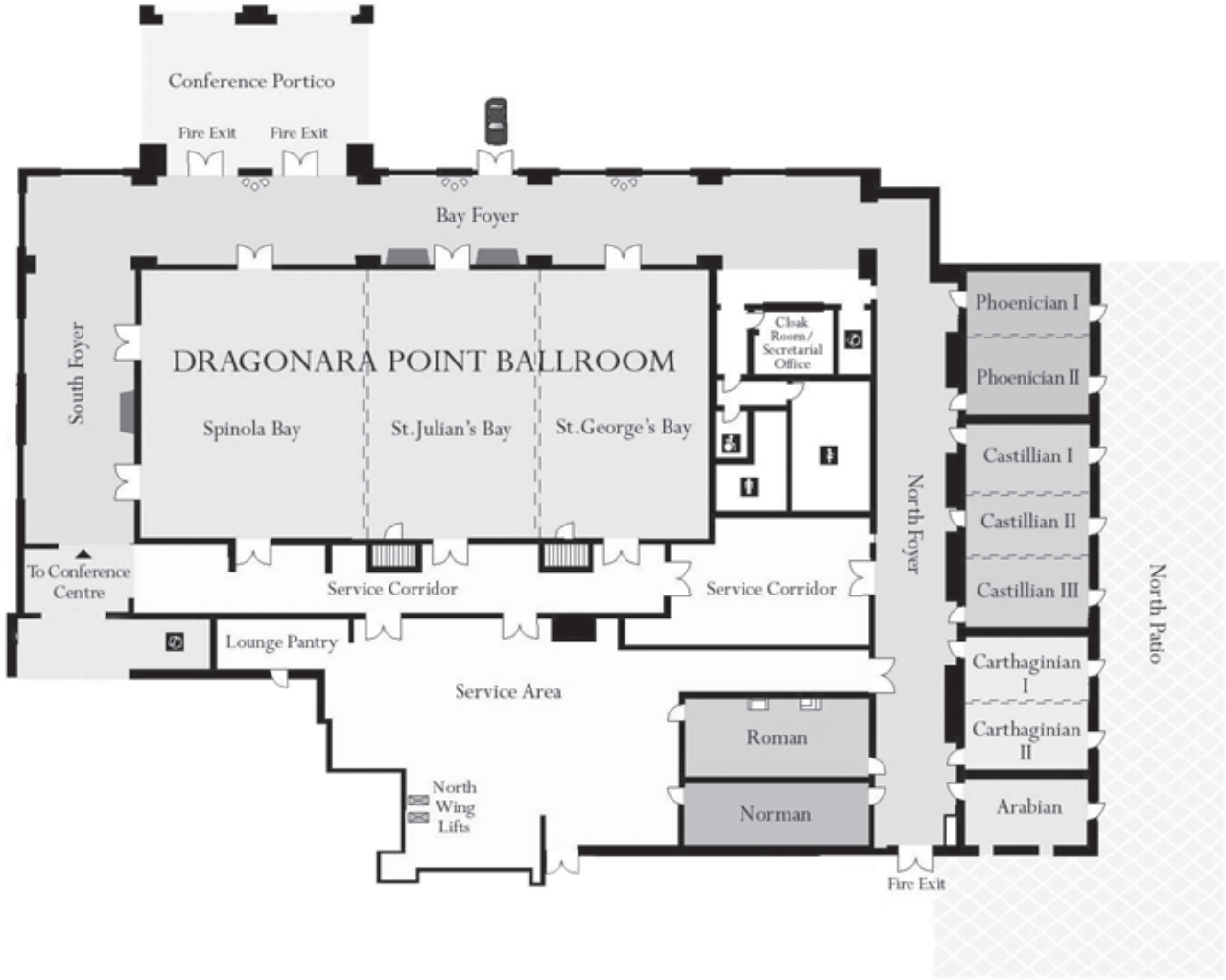
Price Per Person: $39.00 USD
This price includes transportation and entrance into the Casa Rocca Piccola and Palazzo Parisio.

Crafts Village and Meridiana

Tuesday, 9 May 2006 – 9:00
Participants will travel to Ta' Qali at the center of the island, where the "Crafts Village" is located. At this handicrafts center of Malta, a glass factory, a pottery and a filigree artist's workshop with be toured. The second leg of this half-day tour will take guests to the Meridiana wine estate. This 17 hectare vineyard produces exceptional wines, which is supported by the famous Italian wine house of Antinori. The vintner will give a tour of the estate and finish with a wine and cheese tasting.

Price per person: $28.00 USD
This price includes transportation and entrance to the Meridiana Wine Estate.

Conference Center Floor Plan



Contact Information

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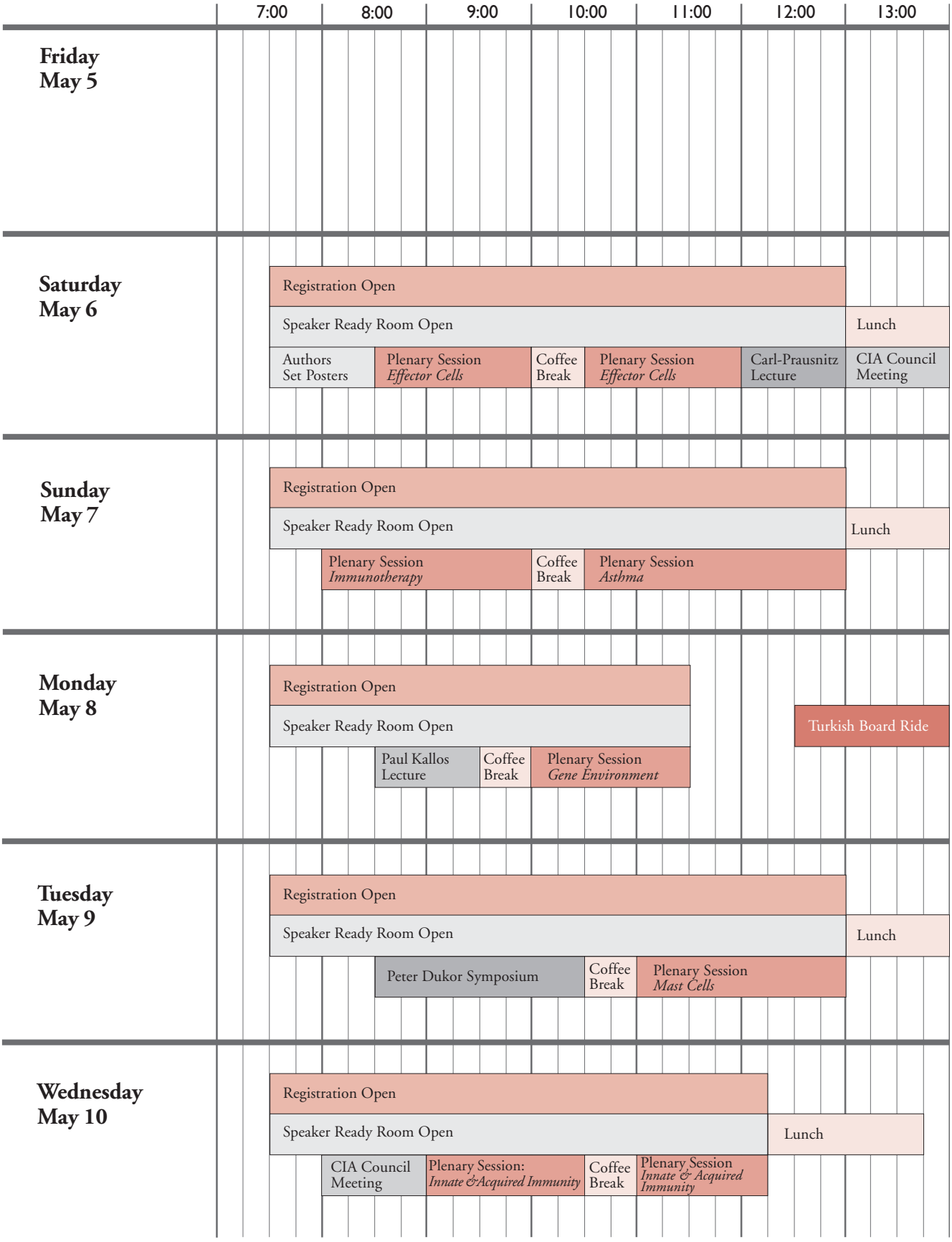
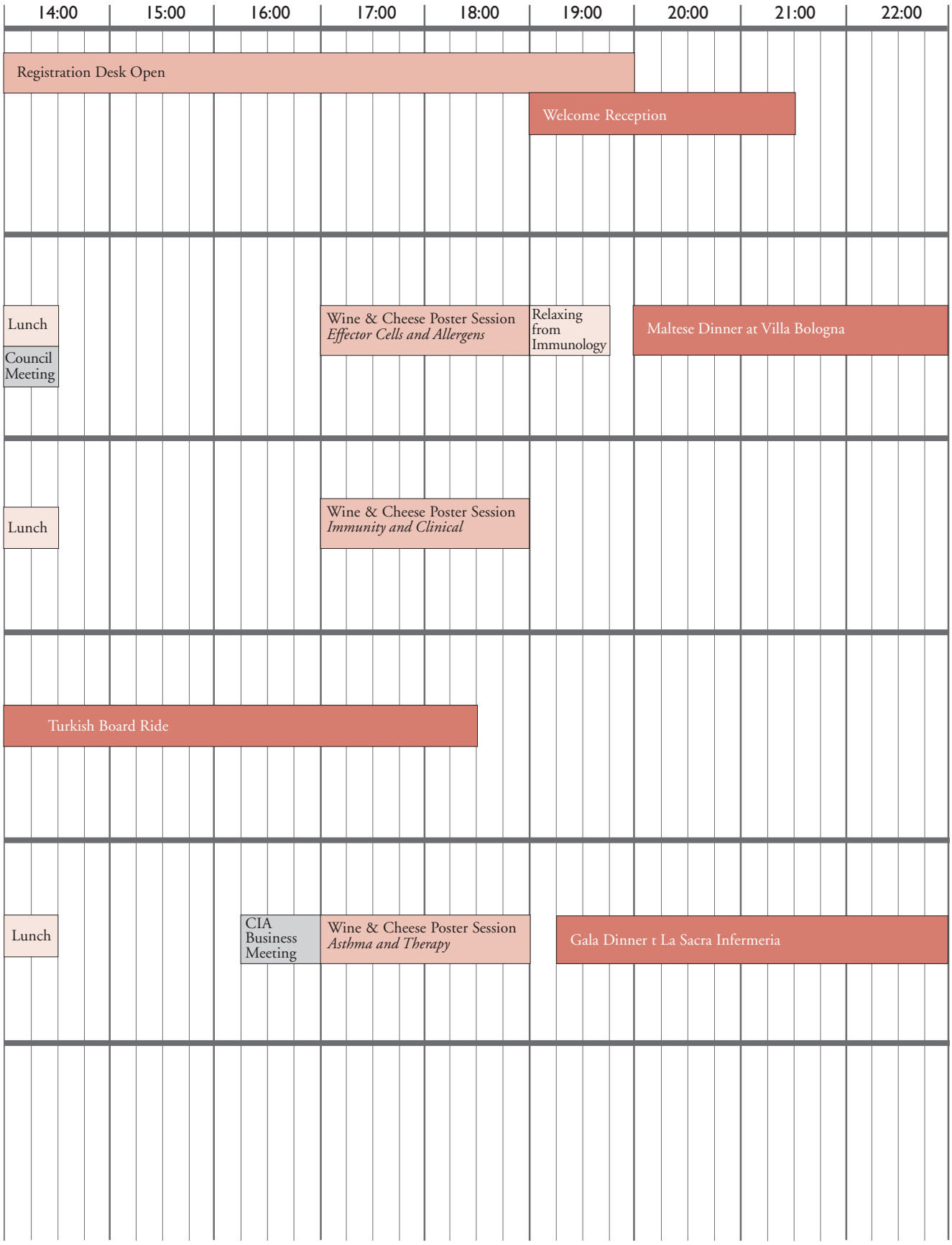
Program at a Glance



Program at a Glance



Schedule of Events

Friday, 5 May 2006

14:00 – 20:00 Registration Open

19:00 – 21:30 **Welcome Reception** — **Westin Dragonara Resort Reef Club**
Stephen Holgate
Barry Kay
Johannes Ring

Saturday, 6 May 2006

7:30 – 8:30 **Authors Set Posters** — **Phoenician, Castillian & Carthaginian Rooms**

7:30 – 13:00 Registration Open

7:30 – 13:00 Speaker Ready Room Open

8:30 – 10:00 **Plenary Session: Effector Cells** — **Dragonara Point Ballroom**
Moderators: John Bienenstock
Dean Metcalfe

1 Pharmacologic regulation of eosinophil activation: new therapeutic targets
Monique Capron

2 Expression and function of vascular endothelial growth factor and its receptors in human basophils
Gianni Marone

3 Surface expression, inhibitory function, and candidate ligand for Siglec-8 on human mast cells
Bruce Bochner

4 Mas-related gene (MRG) receptors and mast cell-nerve interactions
Bradley Undem

10:00 – 10:30 Coffee Break

10:30 – 12:00 **Plenary Session: Effector Cells** — **Dragonara Point Ballroom**
Moderators: Stephen J. Galli
Francesca Levi-Schaffer

5 Calcitonin gene-related peptide in late-phase allergic reactions
A. Barry Kay

6 Human mast cells selectively produce large amounts of CXCR3 ligands
Jean S. Marshall

7 Release of mast cell carboxypeptidase into the circulation in mastocytosis and anaphylaxis
Andrew F. Walls

8 Molecular markers of eosinophilopoiesis at birth: intimations of future atopy and inflammation
Judah A. Denburg

12:00 – 13:00 **Carl-Prausnitz Lecture** — **Dragonara Point Ballroom**
Chair: Stephen Holgate
Johannes Ring: *From wheals and wheeze to tolerance: allergy quo vadis?*
Director and Chairman, Department of Dermatology and Allergy
Technische Universität München

13:00 – 14:30 **CIA Council Meeting** — **Norman Room**

13:00 – 14:30 Lunch — Terrace Restaurant

Saturday, 6 May 2006 (Continued)

17:00 – 19:00 **Wine & Cheese Poster Session** **Phoenician, Castillian & Carthaginian Rooms**

Effector Cells

Moderators: Susan M. MacDonald
Hans-Uwe Simon

9 Selective activation of human mast cells by Escherichia coli hemolysin
Stephan C. Bischoff

10 Determinants of allergen-induced effector cell degranulation: therapeutic inhibition of allergen-induced basophil degranulation with allergen-derived haptens
Anna Gieras

11 The functional role of hepatocyte growth factor in allergic inflammation
Wataru Ito

12 In vivo T helper cell polarizing capacity of pollen-associated lipid mediators (PALMs) during primary sensitization
Thilo Jakob

13 Positive and negative regulation of mast cell activation by Lyn via the FceRI
Toshiaki Kawakami

14 Airway and bone marrow eosinopoiesis after airway allergen exposure; role of eotaxin-2
Jan Lötvall

15 Is human IgE+ equivalent to mouse highly cytokinergic IgE?
Susan MacDonald

16 Protective effect of corm -3, a water soluble carbon monoxide releasing molecule, in a model of vascular inflammation.
Pier Francesco Mannaioni

17 Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites
Salaheddine Mécheri

18 Human mast cells release oncostatin M specifically on contact with activated T Cells
Yoseph Mekori

19 Identification of nitric oxide regulated genes with potential roles in signaling in mast cells
Dean Metcalfe

20 Regulation of human mast cell survival after aggregation of FcγRI or FcεRI
Gunnar Nilsson

21 Role of the high-affinity IgE receptor (FcεRI) beta-chain as a bifunctional signal regulator and molecular mechanisms for regulation of its gene expression.
Chisei Ra

22 Control of exaggerated allergic inflammation through cleavage of IgE by effector cell-derived proteases
Ingrid Rauter

23 Mechanisms of inhibition of human allergic Th2 immune responses by regulatory T cells induced by interleukin 10-treated dendritic cells and transforming growth factor beta.
Joachim Saloga

24 Extracellular traps: A new mechanism used by eosinophils to fight against bacteria
Hans-Uwe Simon

25 Assembly, dynamics and activity of human membrane IgE
Luca Vangelista

Schedule of Events

Saturday, 6 May 2006 (Continued)

26 Caspase activation and loss of mitochondrial membrane potential precedes phosphatidylserine exposure in CD45-dependent eosinophil apoptosis
Garry Walsh

27 Gene expression in the middle ear of mast cell deficient mice
Stephen Wasserman

28 Naive CD4+ T Cell activation by antigen-presenting airway eosinophils
Peter Weller

29 Baseline and stimulated turnover of cell surface c-Kit expression in different types of human mast cells
Torsten Zuberbier

Allergens
Moderators: Kent T. HayGlass
Ronald Van Ree

31 Purification of the natural peanut allergen Ara h 1
Wolf-Meinhard Becker

32 Apples can drive birch pollen-allergic patients nuts! Immunological cross-reactivity as basis for food allergy
Barbara Bohle

33 Mass spectrometric analysis of natural and recombinant preparations of pollen (birch, grass, olive) and house dust mite allergens
Peter Briza

34 Evidence for high Bet v 1 concentrations in ambient air in another compartment than birch pollen?
Jeroen T Buters

35 Nitration of allergens: Another pollution effect to worry about
Albert Duschl

36 Artemisia and Ambrosia hypersensitivity: co-sensitization or co-recognition? A study using recombinant weed pollen allergens
Fatima Ferreira

37 Identification of the major components of the dust mite proteome and the full repertoire of its allergens
Fook Tim Chew

38 Cloning of parvalbumin isoforms from codfish (Gadus morhua) and frog (Rana esculenta)
Karin Hoffmann-Sommergruber

39 Critical assessment of interchangeability of cross-reactive food allergens in diagnostic tests for specific IgE
Jonas Lidholm

40 Determination of the B-cell epitope of hyaluronidase (Hya), a major bee venom allergen, from the crystal structure of Hya in complex with a Fab fragment of a monoclonal antibody.
Zora Markovic-Housley

Schedule of Events

Saturday, 6 May 2006 (Continued)

41 Recombinant hybrid molecule consisting of rPhl p 1, 2, 5 and 6: is it the best combination for grass pollen diagnonis in a group of French patients?
Gabrielle Pauli

42 Epitope mapping and characterization of the binding specificity of monoclonal antibodies directed against allergens of grass group 1
Arnd Petersen

43 A strategy to discriminate between IgE responses to cross-reactive carbohydrate determinants and allergen-specific epitopes
Maryam Poorafshar

44 Relevance of carbohydrate determinants for the differentiation between true latex allergy and asymptomatic IgE reactivity
Monika Raulf-Heimsoth

45 The molecular basis of IgE-mediated autoreactivity
Crameri Reto

46 Acid-suppresssing drugs applied to pregnant females sensitize the mother and support a perinatal Th2-environment in the offspring of BALB/c mice
Isabella Schoell

47 Vacuolar serine protease from Cladosporium herbarum and Alternaria alternata
Birgit Simon-Nobbe

48 Cloning of new Dermatophagoides pteronyssinus allergens for diagnosis and therapy of house dust mite
Susanne Vrtala

49 Prostate-specific antigen as allergen in human seminal plasma allergy
Stephan Weidinger

19:00 – 19:45 **Relaxing from Immunology (guests included)** **Dragonara Point Ballroom**
Chair: Barry Kay
Charles Savona-Ventura: *History of Medicine in Malta*
Senior Lecturer in the Institute of Health Care
University of Malta

20:00 – 23:00 **Maltese Dinner** **Villa Bologna**
Shuttles with guides will depart from the Westin Dragonara Resort at 20:00

Sunday, 7 May 2006

7:30 – 13:00 Registration Open

7:30 – 13:00 Speaker Ready Room Open

8:00 – 10:00 **Plenary Session: Immunotherapy** **Dragonara Point Ballroom**
Moderators: Sabina Rak
Dale T. Umetsu

50 Mechanisms of immune tolerance to high dose allergen exposure in healthy individuals
Mübeccel Akdis

51 Evaluation of safety and clinical efficacy of a higher dose regimen of a ragweed Amb a 1 immunostimulatory conjugate for treatment of patients with ragweed-induced seasonal allergic rhinitis
Peter Creticos

Schedule of Events

Sunday, 7 May 2006 (Continued)

52 Peptide therapy reduces allergic lung inflammation through the induction of IL-10 secreting CD4+ T cells and through deletion of peptide-specific effector T cells in an HLA-DR1 transgenic mouse model
Mark Larche

53 Longitudinal study of sublingual immunotherapy with SQ standardized grass allergen tablets - Interim efficacy results
Henning Lowenstein

54 Induction of "regulatory" T cells by Cynodon dactylon (Bermuda grass) specific immunotherapy
Robyn O'Hehir

10:00 – 10:30 Coffee Break

10:30 – 13:00 Plenary Session: Asthma **Dragonara Point Ballroom**
Moderators: Sven-Erik Dahlen
A. Barry Kay

55 Systemic glucocorticoid fails to inhibit the c-jun/c-jun kinase phosphorylation cascade in bronchial mucosal cells of glucocorticoid resistant asthmatics
Chris Corrigan

56 Association between persistent wheeze and specific IgE, IgG and IgG4 antibodies
Adnan Custovic

57 Mechanistic studies of tumour necrosis factor alpha in asthma
Stephen Holgate

58 Mechanotransduction of the UPA-UPAR in airway epithelial cells
Jeffrey Drazen

59 Helminth infection induces regulatory T cells and inhibits allergen-mediated sensitization and airway disease in a murine asthma model
Eckard Hamelmann

60 CD48 is critically involved in experimental asthma
Francesca Levi-Schaffer

61 The role of NKT cells in the development of asthma
Dale T Umetsu

13:00 – 14 :30 Lunch Terrace Restaurant

17:00 – 19:00 Wine & Cheese Poster Session **Phoenician, Castillian & Carthaginian Rooms**
Immunity
Moderators: Cezmi A. Akdis
Gerhard Schultze-Werninghaus

62 IgE expression is regulated by alternative polyadenylation
Gertrude Achatz-Straussberger

63 Insufficient T regulatory cell expression and function in atopic dermatitis skin
Cezmi A. Akdis

64 Diminished stimulatory capacity of dendritic cells as a result of tryptophan depletion
Thomas Bieber

65 Newly produced immature B cells increase in the lung after airway allergen exposure
Apostolos Bossios

66 Costimulation via CD2 upregulates regulatory T cell associated Foxp3
Malin Fagerås Böttcher

Schedule of Events

Sunday, 7 May 2006 (Continued)

67 Regulatory natural killer cells suppress antigen-specific T cell responses
Gunnur Deniz

68 Immuno-modulatory effects of viral Toll-like receptor ligands: An experimental approach to the hygiene hypothesis
Holger Garn

69 Peanut specific cytokine responses in non-allergic humans are dominated by T cell-dependent Th2 in the absence of detectable Th1 expression.
Kent T. HayGlass

70 Interleukin-4 regulates the expression of thymus - and activation - regulated chemokine/CCL17 by a signal transducer and activator of transcription 6-dependent mechanism
Jutta Horejs-Hoeck

71 Plasma cell differentiation and immunoglobulin-secretion induced by interleukin-4 and anti-CD40 differs in cord blood and adult naïve B cells
Lone Hummelshoj

72 Development of Treg, Th1 and Th2-like immunity during the first 2 years of life in relation to allergic disease
Camilla Janefjord

73 Endotoxin-induced cytokines from antigen-presenting cells during the first two years of life in Estonian and Swedish children
Maria Jenmalm

74 Regulation of dendritic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3
Yuko Kawakami

75 Peroxisome-proliferator activated receptor gamma- and Toll-like receptor-2 agonists might have therapeutical value in Respiratory Syncytial Virus (RSV) induced airway disease
Wolfgang König

76 Molecular mechanism of Th1,Th2 imbalance and hygiene hypothesis
Naomi Kondo

77 Monocyte response to LPS and LTA in allergy
Anna Lindström

78 Long-lived plasma cells and their contribution to allergen-specific IgE
Elke O. Luger

79 Assessment of allergen-specific T cell responses in humans with HLA-class II peptides tetramer technology
Philippe Moingeon

80 T cell regulation of neutrophilic inflammation
Werner Pichler

81 Free immunoglobulin light chains (FLC) in hypersensitivity reactions: need for crosslinking by antigens
Frank Redegeld

82 Macrophage-derived interleukin-10 controls the inflammatory response mediated by TLR-4 but not the response to TLR-9 ligation
Axel Roers

83 M-DC8+ blood dendritic cells are principal producers of early interleukin-12 and are tightly controlled by contact with erythrocytes
Knut Schäkel

Schedule of Events

Sunday, 7 May 2006 (Continued)

84 Reduced allergic airway inflammation and lack of airway hyperresponsiveness (AHR) following sensitisation with dendritic cells over-expressing IL-10
Juergen Schwarze

85 Dermal fibroblast potently induce maturation of dendritic cells (DC)
Jan C. Simon

86 Creating artificial allergens and antibodies for immunotherapy
Beda Stadler

87 NKT cell triggered IL-21 production induced selective Bε cell apoptosis and suppression of IgE responses in mice and humans
Masaru Taniguchi

Clinical
Moderators: Franklin Adkinson
Thomas Bieber

88 Relationship between human anti-chimeric antibodies to infliximab in relationship to infusion related allergic reactions in patients with rheumatoid arthritis
Rob Aalberse

89 Risk assessment for elderly to develop sensitization to respiratory and food allergens
Noemi Bakos

90 A novel function of nerve growth factor: A regulatory role in mucus
Sergio Bonini

91 Chronic Chlamydia pneumoniae infection promotes atherosclerosis by inducing an IL-4-dependent hypersensitivity reaction
Robert Clancy

92 Further considerations on the mechanisms of NSAID hypersensitivity
Alain De Weck

93 Environmental prenatal factors of allergy in Lithuania
Ruta Dubakiene

94 C3a and C4a: Complement split products identify patients with acute - lyme disease
M. M. Glovsky

95 Late onset anaphylactic reactions to Bacillus natto-fermented soybeans, which is well-known as "Natto" of traditional food in Japan
Zenro Ikezawa

96 Single nucleotide polymorphisms of CD14 is associated with the development of respiratory syncytial virus bronchiolitis in Japanese children
Yuzaburo Inoue

97 Predictive value of Phadiatop infant at 2 years for allergic sensitisation at 10 years of age.
Karin C. Lødrup Carlsen

98 Hymenoptera venom allergy: Analysis of double positivity to honey bee and Vespula venom by estimation of specific IgE to species specific major allergens Api m1 and Ves v5.
Ulrich Müller

99 Tropomyosin in invertebrates: Role in IgE crossreactive antibody responses
L Karla Arruda

100 Low levels of plasma retinol in early infancy associate with subsequent development of atopic manifestations
Annamari Ranki

Schedule of Events

Sunday, 7 May 2006 (Continued)

101 Inflammatory response in acute viral exacerbations of COPD
Gernot Rohde

102 Tolerability of imipenem in patients with immediate hypersensitivity to penicillins
Antonino Romano

103 Kinetic study of the antigen-specific expression of lymphocyte activation markers CD69, CD25 and HLA-DR in patients with immediate reactions to Amoxicillin
María L. Sanz

104 Autoreactivity in atopic dermatitis due to molecular mimicry with fungal allergens
Peter Schmid-Grendelmeier

105 Comparison of microarray-based IgE profiling with established diagnostic methods in Patients with latex
Claudia M. Schröder

106 Epidermal caspase-3 cleavage associated with interferon-gamma expressing lymphocytes in acute atopic dermatitis lesions
Dagmar Simon

107 Eosinophilic esophagitis: Escalating epidemiology?
Alex Straumann

108 A geriatric murine model of food allergy
Eva Untersmayr

109 Sensitisation to different pollens and allergic disease in 4 year old Swedish children
Marianne van Hage

110 More than 50% of positive challenges with foods are associated with late eczematous reactions in atopic
Thomas Werfel

111 Delineation of IL-13 effects in skin inflammation of atopic dermatitis
Miriam Wittmann

112 Ten years experience with epidermal dendritic cell phenotyping as a diagnostic tool
Andreas Wollenberg

Monday, 8 May 2006

7:30 – 11:30 Registration Open

7:30 – 11:30 Speaker Ready Room Open

8:30 – 9:30 **Paul Kallos Lecture** **Dragonara Point Ballroom**
Chair: Johannes Ring
Sir Ravinder Maini: *Identification of TNF as a Therapeutic Target*
Professor Sir Ravinder Nath "Tiny" Maini, recently retired as director of the Kennedy Research Institute at Imperial College London. He led a team of researchers over a 15-year period looking for new treatments for rheumatoid arthritis. He is the co-recipient of the 2003 Albert Lasker Award for Clinical Medical Research.

9:30 – 10:00 Coffee Break

10:00 - 11:30 **Plenary Session: Gene Environment** **Dragonara Point Ballroom**
Moderators: Stephen T. Holgate
Heidrun Behrendt

Schedule of Events

Monday, 8 May 2006 (Continued)

113 Detailed sequence and haplotype analysis of the beta-2 adrenergic receptor gene in Caucasians and African Americans
Eugene Bleecker

114 Gene-by-gene interactive for asthma: Use of family studies to identify linked regions
Deborah Meyers

115 Maternal smoking in pregnancy is associated with suppression of neonatal TLR-mediated microbial responses and this effect is increased by maternal allergy.
Susan Prescott

116 Th2 micromilieu generated by pollen associated lipid mediators (PALMs)
Claudia Traidl-Hoffmann

12:30 – 18:30 **Turkish Boat Ride (includes lunch and swimming)**
Shuttles will depart from the Westin Dragonara Resort at 12:30

Tuesday, 9 May 2006

7:30 – 13:00 Registration Open

7:30 – 13:00 Speaker Ready Room Open

8:30 – 10:30 **Peter Dukor Symposium** **Dragonara Point Ballroom**
Moderators: K. Frank Austen
Andrew Saxon

Introductory Lecture
K. Frank Austen: *Innate and adaptive immune functions of the cysteinyl leukotrienes*
AstraZeneca Professor of Respiratory and Inflammatory Diseases
Harvard Medical School
Director, Inflammation & Allergic Diseases Research Section
Brigham and Women's Hospital

117 Co-stimulation of mast cells via FcεRI and Toll-like receptors markedly augments production of inflammatory cytokines
Michael A. Beaven

118 Allergo-oncology: the role of IgE in tumor defense.
Erika Jensen-Jarolim

119 Conformational change in the IgE-FcεRI interaction as a target for inhibitor design
Brian Sutton

120 IgE switching and synthesis is regulated locally in the bronchial mucosa in atopic and non-atopic asthma
Pooja Takhar

10:30 – 11:00 Coffee Break

11:00 – 13:00 **Plenary Session: Mast Cells** **Dragonara Point Ballroom**
Moderators: Gianni Marone
Hirohisa Saito

121 Mast cells-progenitors are recruited to lung following induction of allergic inflammation and this is mediated by alpha4 Integrins, CXCR2 and VCAM-1
Michael Gurish

122 Evaluation of the function of mast cell tryptases using recombinant material and novel transgenic mice.
Roberto Adachi

Schedule of Events

Tuesday, 9 May 2006 (Continued)

124 Integrating control of mast cell function and allergic responses
Juan Rivera

125 RabGEF1 is a negative regulator of Ras signaling and FcepsilonRI- or c-Kit-dependent activation in mast cells in vitro, and of mast cell-dependent biological responses in vivo.
Stephen Galli

126 Syk, but not SHIP1, expression regulates IgE-mediated responsiveness of human basophils
Donald MacGlashan

127 Gene expression profiling of human mast cell lines
Hirohisa Saito

13:00 – 14:30 Lunch — Terrace Restaurant

16:15 – 17:00 CIA Business Meeting — **Dragonara Point Ballroom**

17:00 – 19:00 Wine & Cheese Poster Session — **Phoenician, Castillian & Carthaginian Rooms**

Asthma
Moderators: Tak Lee
Alkis Togias

128 Positive Phadiatop infant at 2 years increases the risk of asthma at ten years
Kai-Håkon Carlsen

129 The impact of inflammation and angiogenesis on airway mucosal blood flow in asthma
Graham Clarke

130 Hospital admission with acute asthma exacerbation, specific IgE quantification and virus infection
Adnan Custovic

131 Tumor necrosis factor-related apoptosis inducing ligand is a key regulator of Th2 cell responses and allergic disease of the lung
Paul Foster

132 Reversing the defective induction of interleukin-10 secreting T cells in glucocorticoid resistant asthma
Catherine Hawrylowicz

133 IgE against Staphylococcus aureus enterotoxins and asthma severity
Peter Howarth

134 Involvement of periostin in subepithelial thickening of bronchial asthma downstream of IL-4 and IL-13
Kenji Izuhara

135 Expression and function of thymic stromal lymphopoietin (TSLP) in allergic rhinitis patients
Frode Jahnsen

136 Essential role of interferon-γ and Th1 immune response in the pathogenesis of non-eosinophilic asthma
You-Young Kim

137 Allergen-induced asthma-like reaction in sensitized guinea pigs was reduced by pretreatment with epigallocatechine-3-gallate
Emanuela Masini

138 Interaction of allergic rhinitis and bacterial sinusitis in a mouse model
Robert Naclerio

Schedule of Events

Tuesday, 9 May 2006 (Continued)

139 Role of IL-5, eosinophils and TGF-β1 in allergen-induced subepithelial fibrosis in mice
Hiroichi Nagai

140 Induction and efficacy of cytolytic regulatory T cells in experimental asthma
Jean-Marie Saint-Remy

141 Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy
Glenis Scadding

142 Pro-angiogenic properties of bronchoalveolar lavage fluid from asthmatics
David Simcock

143 Aspirin sensitive rhinosinusitis is associated with reduced E-prostanoid 2 (EP2) receptor expression on nasal mucosal inflammatory cells
Ying Sun

144 Effects of lipid mediators on the activation of human lung fibroblasts
Shigeru Takafuji

145 Epidemiology of the allergic respiratory syndrome in the residents of urban US public housing
Alkis Togias

146 Chymase polymorphism is associated with the severity of atopy in asthmatics
Andrew F Walls

147 Lymphocyte migration to the lung
Andrew Wardlaw

148 IgE dependent cytokine release from human lung tissue.
Jane Warner

149 Role of advanced glycation end-products in lung disease
Susan Wilson

Therapy
Moderators: Stephen Durham
Yoseph A. Mekori

150 Modulation of the IgE response by blocking membrane-IgE
Gernot Achatz

151 Sublingual immunotherapy reduces allergic symptoms in a mouse model of rhinitis
Jens Brimnes

152 Anaphylactic and anaphylactoid reactions to paclitaxel, carboplatin and doxorubicin: treatment with rapid out-patient desensitization.
Mariana Castells

153 Intramuscular immunization with DNA construct containing Der p 2 and signal peptide sequences primed strong IgE production
Kaw Yan Chua

154 Time course analysis of clinical and immunological markers of tolerance during grass pollen immunotherapy
James Francis

155 Directed molecular evolution of mite group 2 allergen genes generating hypoallergens for allergen-specific immunotherapy
Guro Gafvelin

Schedule of Events

Tuesday, 9 May 2006 (Continued)

156 Adsorption of allergens to aluminium hydroxide adjuvant induces only minor or no structural changes of the allergen
Charlotte Hejl

157 Development of a mucosal polyvalent allergy vaccine for primary prevention of multi-sensitization
Karin Hoffmann-Sommergruber

158 IgE-regulatory effects of histamine in atopy and non-atopic diseases
R.A. Khanferyan

159 Antibody responses to minor allergen Bet v 2 during allergen specific immunotherapy in birch pollen allergic
Jørgen Nedergaard Larsen

160 Alpha melanocyte stimulating hormone and fragments: potential therapeutic agents in inflammation
T.A. Luger

161 Discovery of LAS 36674, a new generation of H1 antihistamines: from bench to bedside and back
Montse Miralpeix

162 A potent adenosine A2B receptor antagonist attenuates methacholine-induced bronchial hyperresponsiveness, mucus production and IgE levels in an allergic mouse model.
Arsenio Nueda

163 Vaccination with genetically modified birch pollen allergens has beneficial effects on birch pollen allergy-associated oral allergy syndrome
Verena Niederberger

164 Clinical and immunological changes in atopic dermatitis patients undergoing subcutaneous immunotherapy with house dust mite allergens
Natalija Novak

165 Induction of allergen-specific CD8+ T cells with protective effects against allergic airway inflammation
Markus Ollert

166 Hymenoptera venom allergy: A new ultra-rush immunotherapy
Vincenzo Patella

167 A follow-up study of immunotherapy-treated birch-allergic patients. The effect on expression of chemokines in nasal biopsies.
Sabina Rak

168 Pharmacodynamics of latest generation H1-antihistamines: relevance of drug concentrations at receptor sites and of affinity values for H1 receptors
Margherita Strolin Benedetti

169 Development of a vaccine for the treatment of fish allergy
Ines Swoboda

170 Anti-IgE treatment overcomes intolerability of honeybee-venom ultra-rush immunotherapy in indolent systemic mastocytosis
Thomas Werfel

171 A hypoallergenic vaccine obtained by tail-to-head restructuring of timothy grass pollen profilin, Phl p 12, for the treatment of cross-sensitization to profiling
Kerstin Westritschnig

Schedule of Events

Tuesday, 9 May 2006 (Continued)

172 Screening for cellular changes during grass-specific subcutaneous immunotherapy
Peter Adler Würtzen

173 Allergenicity and immunogenicity of allergoid products commercially available for birch pollen
Hendrik Wolf

19:15 – 23:00 **Gala Dinner** **La Sacra Infermeria**
Shuttles will depart from the Westin Dragonara Resort at 19:15

Wednesday, 10 May 2006

8:00 – 9:00 **CIA Council Meeting** **Norman Room**

7:30 – 12:15 Registration Open

7:30 – 12:15 Speaker Ready Room Open

9:00 – 10:30 **Plenary Session: Innate and Acquired Immunity** **Dragonara Point Ballroom**
Moderators: Alain L. de Weck
Takemasa Nakagawa

174 Integration of regulatory T cells into the Th1/Th2 paradigm
Carsten Schmidt-Weber

175 Group V phospholipase A2 and innate immunity
Jonathan Arm

176 Biphasic itch stimulus model for investigations using functional magnetic resonance tomography (fMRT)
Ulf Darsow

177 Novel genes distinguishing inhalant allergen-specific T-cell memory responses in atopics
Patrick Holt

10:30 – 11:00 Coffee Break

11:00 – 12:15 Plenary Session: Innate and Acquired Immunity **Dragonara Point Ballroom**
Moderator: Mübeccel Akdis
Johannes Ring

178 CD8 T cells stimulate dendritic cells to secrete IL-18 that induces Th1 and inhibits Th2 cell differentiation in vitro and suppresses IgE responses in vivo
Mike Kemeny

179 Characterization of the structure and proteolytic activity of the major house dust mite allergen Der p 1
Kåre Meno

180 Plasticity of histamine H1 receptor during human macrophage differentiation
Massimo Triggiani

12:15 – 13:45 Lunch Terrace Restaurant

Abstracts Listing

1

Pharmacologic regulation of eosinophil activation: new therapeutic targets

David Dombrowicz, Monique Capron, Delphine Staumont, Francois Trottein

Inserm U547, Institut Pasteur de Lille, France.

Eosinophils are associated to various allergic diseases, in particular asthma and atopic dermatitis. In order to investigate new potential therapeutic targets, we examined the role of Prostaglandin (PG) D2 and Peroxisome Proliferator-Activated Receptors (PPAR) in the regulation of eosinophilia in mouse models of allergen-induced asthma and atopic dermatitis mimicking the human pathology. PGD2 is an arachidonic acid derivative mainly produced by mast cells and binding to two receptors with broadly antagonistic properties: DP1, an inhibitory receptor, and DP2/CRTH2, a stimulatory receptor. PPAR are ligand-activated transcription factors existing under 3 isoforms: -α, -β/-δ and -γ and forming dimers with the retinoic acid X receptor (RXR). PPAR have been shown to regulate an increasing number of processes within the immune system.

Using specific agonists for DP1, DP2, PPAR-α, -β/-δ and -γ as well as PPAR-α-deficient animals, we could demonstrate that DP1 activation inhibited, while DP2 activation promoted, the development of eosinophilia in both atopic dermatitis and asthma. These changes were associated to a decreased/increased pathology (including epidermal thickening, airway hyperresponsiveness, IgE production and cellular inflammation). These results lead us to propose to use DP1 agonists or DP2 antagonists to prevent allergy-associated eosinophilia.

Using a similar approach with PPAR agonists, we found that both PPAR-γ efficiently inhibited allergic asthma and airway remodelling but was without effect in atopic dermatitis. By contrast, PPAR-β/-δ most efficiently decreased skin inflammation. Finally, PPAR-α agonists and PPAR-α-deficient animals provided mirror-images in both pathologies, where agonists inhibited disease development and deficient animals displayed a worsening of the symptoms. Taken together, our results suggest that according to the tissue localization of the disease, PPAR isoforms might exert differential effects.

In conclusion, both PGD2 receptors and PPAR could be considered as interesting new therapeutic targets for eosinophil-associated diseases.

2

Expression and Function of Vascular Endothelial Growth Factor and its Receptors in Human Basophils

Amato de Paulis[1], Nella Prevete[1], Isabella Fiorentino[1], Francesca W. Rossi[1], Bianca Liccardo[1], Domenico Ribatti[2], Andrew F. Walls[3], Stefania Staibano[4] and Gianni Marone[1]

[1] Department of Clinical Immunology and Allergy, University of Naples Federico II, Naples, Italy

[2] Department of Anatomy, University of Bari, Bari, Italy

[3] Immunopharmacology Group, Southampton General Hospital, Southampton, United Kingdom

[4] Department of Pathology, University of Naples Federico II, Naples, Italy

Angiogenesis is a multiple complex phenomenon that is critical for several chronic inflammatory disorders such as bronchial asthma and tumor growth. Vascular endothelial growth factor (VEGF) is the most potent proangiogenic mediator. The VEGF family consists of several members, including VEGF-A, VEGF-B, VEGF-C, and VEGF-D. VEGF-A and -B are key regulators of blood vessel growth, whereas VEGF-C and -D regulate lymphatic angiogenesis. Some of these family members have differentially spliced forms with a range of abilities to enhance angiogenesis. Human basophils express three isoforms of mRNA for VEGF-A (VEGF-A_{121}, $_{165}$ and $_{189}$) and two isoforms of VEGF-B. mRNA for VEGF-C and VEGF-D were not detected. Basophils contain 148 ±10.1 pg/10^6 cells of VEGF-A. By confocal microscopy VEGF-A was co-localized within secretory granules of peripheral blood basophils and basophils infiltrating nasal polyps. Basophils activated by anti-IgE (0.1 µg/ml) released VEGF-A. By flow cytometric analysis the majority of basophils (~ 70%) expressed two VEGF receptors: VEGFR-1/Flt-1 and VEGFR-2/KDR. Low concentrations of VEGF-A_{165} (30 – 500 ng/ml) and to a lesser extent VEGF-A_{121} caused basophil chemotaxis. Preincubation of basophils with anti-VEGFR-1 and/or anti-VEGFR-2 inhibited VEGF-A- dependent basophil chemotaxis. VEGF-A_{165} did not cause histamine and cytokine (IL-4 and IL-13) release from basophils. Supernatants of anti-IgE-activated basophils induced an angiogenic response in the chick embryo chorioallantoic membrane (CAM). Anti-VEGF antibody reduced the angiogenic response of basophil supernatants.

In conclusion, the results have identified blood basophils as a major source of VEGF-A. VEGF-A induces basophil chemotaxis and angiogenesis in vitro suggesting that basophils play an important role in the control of angiogenesis in chronic inflammatory disorders. These findings emphasize the importance of basophils and their angiogenic factors in the modulation of tissue remodelling and angiogenesis in allergic disease.

3

Surface expression, inhibitory function, and candidate ligand for Siglec-8 on human mast cells

Bruce Bochner, Ronald Schnaar, Nicholai Bovin, Sherry Hudson, Hidenori Yokoi

Siglecs (sialic acid–binding immunoglobulin-like lectins) are a family of innate immune receptors that are transmembrane I-type lectins characterized by the presence of an N-terminal V-set immunoglobulin domain that binds sialic acid. A unique characteristic of many Siglecs is the presence of conserved cytoplasmic sequences containing tyrosine motifs, suggesting that these molecules possess inhibitory functions (e.g., immunoreceptor tyrosine-based inhibitory motifs or ITIMs). Our previous work has focused on characterizing Siglec-8 expression and function on human eosinophils, where activation by antibody crosslinking induces apoptosis. We therefore hypothesized that Siglec-8 crosslinking on mast cells would inhibit their function. Human mast cells were generated from peripheral blood CD34+ precursors using standard culture methods. As expected, CD34+ precursors did not express FcεRI or Siglec-8, but by 4 weeks of culture, cells began to express both FcεRI and Siglec-8 (as detected by immunofluorescence and flow cytometry) in parallel with histamine and tryptase. Siglec-8 surface expression plateaued by 8 weeks. Unlike for eosinophils, antibody crosslinking did not induced mast cell apoptosis, but instead signficantly inhibited FcεRI-dependent release of histamine and PGD2 by approximately 50%.

One of the shortcomings of these studies is that an antibody is used to activate Siglec-8 because its ligand was unknown. However, through the efforts of the Consortium for Functional Glycomics (www.functionalglycomics.org), ligand screening for Siglec-8 was performed and among about 180 carbohydrate ligands, only one, 6'-sulfo-sialyl Lewis X, was identified, with a relative affinity of about 2 µM. Closely related glycans, including 6-sulfo-sialyl Lewis X (an L-selectin ligand) and sialyl Lewis X (a common selectin ligand) did not bind Siglec-8. Subsequently, using biotinylated multivalent polyacrylamide polymers, selective binding of 6'-sulfo-sialyl Lewis X, but not any of these same structurally related glycan polymers, was confirmed in Siglec-8 transfectants using flow cytometry, but do not bind to eosinophils or mast cells, suggesting that glycan confirmation is important for human cell binding of ligand. Taken together, these data suggest that aggregation of Siglec-8, either via a monoclonal antibody or perhaps with an appropriate multivalent form of 6'-sulfo-sialyl Lewis X or glycomimetic, could potentially be used therapeutically to reduce eosinophil numbers and mast cell activation.

4

***Mas* Related Gene Receptors and Mast Cell Nerve Interactions**

Bradley J. Undem, Min-Goo Lee, Oksoon H. Choi, Donald W. MacGlashan Jr., Xinzhong Dong

A family of G-protein coupled orphan receptors has recently been described that are found only in primary sensory neurons (Cell, 106:619-32, 2001). These receptors are referred to as either subtypes of *m*as *r*elated *g*ene (Mrg) receptors or *s*ensory *n*erve *s*pecific" (SNS) receptors. In rats, Mrg A1 and Mrg C11 are G(q)-coupled receptors localized exclusively to nociceptive-type sensory nerves. It has long been known that mast cells are situated in close proximity to nociceptive (C-fiber-type) sensory nerves in skin and visceral tissues. We addressed the hypothesis that mast cells may communicate with these nerves via Mrg receptors. Mrg A1 and Mrg C11 are effectively activated by FMRF-amide and other RF-amide related peptides. In calcium imaging studies, we found that FMRF-amide (1 µM) stimulated 62 of 73 vagal nodose sensory neurons, and 136 of 272 trigeminal sensory neurons. In electrophysiological patch clamp recordings FMRF-amide (1 µM) evoked action potential discharge in the majority of vagal (4 of 6) and trigeminal (3 of 5) neurons. FMRF-amide was immunohistochemically localized to mast cells in mouse skin, and the mRNA for RF-amide propeptide is expressed in RBL-2H3 cells. IgE/antigen stimulation of RBL cells lead to activation of co-cultured HEK cells when the HEK cells were transfected with Mrg C11 receptors. These results provide preliminary circumstantial support for the hypothesis that Mrg receptor agonists may represent a novel class of mast cell mediators involved with allergy-induced sensory nerve activation.

5

Calcitonin gene-related peptide in late-phase allergic reactions

Mark Larche, Tak Lee, Sun Ying, Julia Barkans, Farid Benyahia, Liam Heaney, F.Runa Ali, A. Barry Kay

Late-phase asthmatic and cutaneous reactions (LPRs) provoked under controlled conditions either by whole allergen or allergen-derived T-cell peptides remain useful models for studying the mechanisms of allergic inflammation. However the oedematous component of these reactions remains unclear. We have tested the hypothesis that calcitonin gene-related peptide (CGRP) is involved in LPRs since previous studies in man have shown that this neuropeptide is a potent vasodilator as well as contracting airway smooth muscle. Late asthmatic reactions (LAR) were provoked by aerosol inhalation of allergen-derived T-cell peptide epitopes in 24 cat-allergic subjects of whom 12 (termed "responders") developed LARs 6 hours after inhalation of Fel d 1-derived peptides. Bronchoscopy, with bronchial biopsies and bronchoalveolar lavage (BAL), was performed at 6 hours and measurements made by immunohistochemistry and ELISA. Responders, but not non-responders, had increased airway hyperresponsiveness (AHR) after peptide challenge which was accompanied by significant increases in BAL in the concentration of CGRP, but not substance P or NK-A. Furthermore CGRP concentrations correlated with the changes in AHR. In virtually all responders peptide challenge induced marked increases in CGRP immunoreactivity in bronchial epithelial cells, infiltrating submucosal cells as well as upregulation of positive staining in association with airway smooth muscle. We also studied skin biopsies (n=8) from atopic volunteers challenged at sites challenged with whole allergen and found that CGRP immunoreactivity increased, peaked and declined with the magnitude of the late-phase skin response. Thus, the late-phase allergic response is associated with enhanced CGRP expression supporting the concept that changes in the vasculature is an important component of allergic inflammation in the skin and airways.

6

Human mast cells selectively produce large amounts of CXCR3 ligands

Jean S. Marshall, Sarah M. Burke, Suzanne P Zinn

Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada.

The CXCR3 ligands, CXCL9, CXCL10 and CXCL11 are critical for T-cell recruitment and have been implicated in type 1 responses and viral infection. CXCR3 has also been implicated as an important receptor for the recruitment of mast cells in asthma. We investigated the role of human mast cells as a source of CXCR3 ligands in response to inflammatory cytokines or the double stranded RNA analogue polyinosine-polycytidylic acid (poly(I:C)) as a model of toll like receptor 3 activation during viral infection. Human cord blood derived mast cells (CBMC) produced substantial CXCL9 and CXCL10 following activation with interferon-gamma (IFN-^{3}), alone or in combination with tumour necrosis factor (TNF) or interleukin-1-beta (IL-1^{2}). The production of CXCR3 ligands by mast cells in response to IFN-^{3} was highly selective and was not accompanied by degranulation, granule-macrophage colony stimulating factor (GM-CSF) or CCL5 responses. Production of all three CXCR3 ligands was observed following 6h of IFN-^{3} activation with CXCL9 production sustained for up to 48h. The amounts of CXCL10 produced by human mast cells were 20 -100 fold greater than those reported by other immune effector cells such as eosinophils and neutrophils in response to IFN-^{3}. In keeping with a potential role in responses to virus infection, human mast cells also selectively produced CXCL10 when activated with poly(I:C). These data suggest that human mast cells can be major and sustained sources of CXCR3 ligands. Mast cell production of CXCR3 ligands may be of particular importance at sites of viral infection or IFN-^{3} production where it may enhance further recruitment of mast cells, T-cells and NK cells.

Supported by the Canadian Institutes of Health Research

7

Release of mast cell carboxypeptidase into the circulation in mastocytosis and anaphylaxis

Xiaoying Zhou, Mark G Buckley, Laurie C Lau, Colin Summers, Richard S H Pumphrey, Rosa Nuñez, Aranzazu Prados, Manuela Cuevas, David González, Luis Escribano, Andrew F. Walls

Mast cell tryptase has become a useful clinical marker in cases of mastocytosis as well as in anaphylactic shock. However, increased tryptase levels in the circulation are not an invariable feature and there is a need for more discriminating laboratory tests. We have developed an ELISA procedure for the measurement of mast cell carboxypeptidase using specific monoclonal antibodies, and applied it to determine levels of this unique mast cell product in serum from different categories of mastocytosis and anaphylaxis. Paediatric mastocytosis cases included those with urticaria pigmentosa (19 samples) and diffuse cutaneous mastocytosis (5). Adult cases comprised: pure cutaneous mastocytosis with only skin involvement (3), indolent systemic mastocytosis (31), systemic mastocytosis associated with recurrent anaphylaxis without skin lesions (6), well-differentiated systemic mastocytosis (4), aggressive systemic mastocytosis (7), mast cell leukaemia (1), and systemic mastocytosis associated with haematological non-mast-cell disease (7). In addition, serum was also included from 183 separate cases of anaphylaxis, collected within 8h of the reaction. Control groups included serum collected from healthy blood donors (113) and from patients with chronic urticaria (3) or with bronchial asthma (15). There were no differences between levels in any of the control groups, but carboxypeptidase concentrations were significantly higher in both the paediatric and adult cases of mastocytosis and in the anaphylaxis cases ($p < 0.0001$ for each group). Concentrations of carboxypeptidase were not associated with those of tryptase in any of the mastocytosis or anaphylaxis groups, and an elevated carboxypeptidase level was seen in many cases that were tryptase-negative. An assay for mast cell carboxypeptidase should represent a valuable laboratory test in cases of suspected mastocytosis and anaphylaxis.

Research funded by: Thrasher Research Fund, REMA G03/007; FIS. 03/0770; CAM:GR/SAL/0133/2004, Fundacion MMA.

8

Molecular Markers of Eosinophilopoiesis at Birth: Intimations of Future Atopy and Inflammation.

Judah A. Denburg, MD, FRCPC
Professor of Medicine
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Dysfunctional *adaptive* (T-cell) immunity in the genesis of atopy and asthma is paralleled by abnormalities in *innate* immunity, including the contribution of bone marrow and tissue hemopoietic progenitors, particularly of the eosinophil/ basophil (Eo/B) lineage. We have recently reported that cord blood (CB) Eo/B progenitor cytokine receptors are associated with increased atopic risk, showing an *inverse* correlation between maternal skin prick test responses to common allergens and IL-5R∀/ GM-CSFR∀ expression on CB $CD34^{+}$ cells at birth, and a *positive* correlation with IL-3R∀. These alterations in CB progenitors correlate with clinical outcomes at one year,Percentages of CB $CD34^{+}$ cell numbers were found to be higher after n-3 PUFA than placebo ($p<0.003$). Co-expression of cytokine or chemokine receptors on CD34 cells was not altered by n-3 PUFA supplementation. However, there were significantly more IL-5 responsive, but not IL-3 or GM-CSF responsive, CB Eo/B-CFU in the fish oil group compared to the control group ($p<0.03$), which correlated with clinical outcomes at one year: predicting both *atopic dermatitis* and *wheeze*, and can be modified in response to maternal dietary intervention during pregnancy (Upham et al, JACI 104:370; Denburg et al, Pediatr Res 57:276). Currently, we examined the relationship between CB progenitor function and phenotype in 39 infants from an atopic high-risk Australian birth cohort, and the clinical response to acute respiratory illness (ARI) in the first year. A consistent relationship was observed between increased numbers of GM-CSF- and IL-3-responsive Eo/B-colony-forming cells (CFU) at birth and the frequency and severity of ARI, including accompanying wheeze ($p=0.023$) or fever ($p= 0.013$). Comparable associations were found between ARI and CB IL-3R±$^{+}$ and GM-CSFR±$^{+}$ $CD34^{+}$ cell numbers. Conversely, a reciprocal decrease in the proportion of CB IL-5R±$^{+}$ cells was found in relation to the clinical outcomes. Real-time polymerase chain reaction (Q-PCR) was employed to ascertain the kinetic patterns of expression of Eo/B-lineage specific genes, GATA-1, MBP and IL-5R± in random, fresh and frozen CB samples. Stimulation with IL-5 resulted in an early up-regulation of GATA-1 expression, peaking at 24-48h. In contrast, MBP was up-regulated in a slowly progressive pattern, maximally at 72h, while there was stable, low expression of IL-5R±. Numbers of Eo/B-CFU related to antecedent GATA-1 expression. These results provide a plausible mechanism for the generation of tissue airway eosinophilic and neutrophilic inflammation during ARI, and may explain epidemiological associations between atopy and wheezing in response to viral ARIs. Molecular markers of critical, Eo/B lineage-specific events in CB may herald future atopic and asthmatic biological and clinical outcomes.

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Selective activation of human mast cells by escherichia coli hemolysin

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Mast cells (MCs) are known to play an important role in innate immunity in the murine system. Here, we studied the interaction of human MCs, isolated from cord-blood or the intestine, with different *Escherichia coli* strains, and with toll-like receptor (TLR) ligands (L). MCs were challenged with human fecal *E. coli* isolates, the ATCC strains 25922 and 35218, hemolysin-negative (Hly-) mutants of these strains, FimH+ and FimH- *E.coli* strains, the probiotic *E. coli* Nissle 1917 (Mutaflor®) and a HLy+ mutant of *E.coli* Nissle 1917. TLR expression was analyzed by RT-PCR and flow cytometry. MC were challenged with LTA and Zymosan (TLR2Ls), poly I:C (TLR3L), LPS (TLR4L), R848 (TLR7/8L), and CpG (TLR9L). Cytokine mRNA expression was quantified by real time RT-PCR. Histamine, sulfidoleukotrienes (sLT), TNFα, and IL-8 were measured by immunoassay. In contrast to previous studies performed with rodent and human cord blood-derived mast cells, human intestinal MC did not respond to TLR ligands. They express mRNA for TLR 1, 2, 3, 4, 5, 6, 8, and 9, which could be confirmed at the protein level for TLR 2, 3 and 4. However, stimulation with TLR ligands neither led to elevated mRNA expression nor the release of histamine, TNFα, and IL-8. Preculture of the cells with IL-6, INFγ or IL-4 did not alter MC response towards stimulation by TLRL. In contrast, cord blood derived MC could be activated through TLRL for mediator release., as described before. Most interestingly, we show that α-hemolysin (Hly)-producing E. coli strains, but not isogentic Hly-deficient mutant strains, induce calcium influx and release of histamine, sLT, and cytokines in intestinal MCs. MC activation by Hly+ E. coli was almost abolished by blocking L-type calcium channels with nifedipine. Prolonged infection with Hly+ E. coli resulted in lysis of intestinal MCs indicating a biphasic action of Hly. In summary, our data show that human MCs can be activated by selected gram- E.coli strains. The mechanism of activation is independent of TLR signaling, which is silenced in intestinal MC. Instead, E. coli Hly was identified as a new factor regulating human mucosal MC effector functions.

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Determinants of allergen-induced effector cell degranulation: therapeutic inhibition of allergen-induced basophil degranulation with allergen-derived haptens

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We have used the grass pollen allergen, Phl p 1, a major respiratory allergen for approximately 400 million allergic patients to dissect molecular determinants of allergen-induced effector cell degranulation. A monoclonal murine IgE antibody against a Phl p 1-derived peptide, P1, was generated and used to sensitize rat basophil leukemia (RBL) cells. Using the P1 monomer, a dimer, and a P1 polymer it was demonstrated that the number of IgE epitopes on a given allergen molecule determines the extent of basophil degranulation. The amount of mediators released depended also on the concentration of allergen-specific IgE antibodies used for sensitization. The P1 monomer did not cause any mediator release and prevented basophil degranulation by the P1 polymeric form.

Our results may explain the heterogeneity in allergenic activity of allergen molecules through variation of the numbers of recognized IgE epitopes. Furthermore, they may explain why allergic patients, who have increased their allergen-specific IgE production become more sensitive to the allergens. Finally, allergen-derived haptens, such as P1 monomer, can inactivate allergic effector cells and maybe used for therapy of allergy.

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The functional role of hepatocyte growth factor in allergic inflammation

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Hepatocyte growth factor (HGF) is recognized as a humoral mediator of epithelial-mesenchymal interactions in tissue regeneration and recent studies have reported that HGF prevents lung fibrosis as well as fulminant hepatic failure and liver cirrhosis. In this study, we first investigated the role of HGF in a murine model of allergen-induced airway inflammation. After ovalbumin (OVA) sensitization and airway challenge, airway function was monitored by changes enhanced pause (Penh) to inhaled methacholine. Following OVA challenge, Penh significantly increased as did the number of lung inflammatory cells and Th2 cytokines levels in bronchoalveolar lavage fluid (BALF) ($p<0.05$). Administration of HGF during OVA challenge significantly prevented changes in Penh and eosinophil accumulation in the airways as well as Th2 cytokines levels and eosinophil numbers in BALF ($p<0.05$). On the other hand, neutralization of endogenous HGF significantly developed AHR and airway inflammation ($p<0.05$). Eosinophils play a pivotal role in the mechanism of allergic diseases including bronchial asthma. Next, we investigated the effect of HGF on human eosinophil chemotaxis. Human peripheral blood eosinophil were purified by negative selection method using anti-CD16 immunomagnetic beads. Eosionophils were preincubated with/without HGF at 37 for one hour. Chemotaxis of eosinophils was conducted in Boyden chambers. HGF inhibited the eosinophil chemotaxis towards eotaxin or prostagladin D_2 (PGD_2) in a dose-dependent manner ($p<0.05$). Moreover, we demonstrated that HGF suppressed ERK1/2 and p38 MAPK activation which are indispensable for eosinophil chemotaxis. These data indicate that HGF plays an important role in the development of asthma and treatment with HGF may be considered as a new therapeutic strategy for allergic diseases.

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In Vivo T Helper Cell Polarizing Capacity of Pollen-Associated Lipid Mediators (PALMs) during Primary Sensitization

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Pollen grains not only function as allergen carriers, but also release bioactive lipid mediators (PALMs) upon contact with the aqueous phase. We recently demonstrated that PALMs modulate human dendritic cells function in a fashion that results in a Th2 polarization of human naïve T cells *in vitro*.(JEM 201:627-635). To address, whether PALMs show similar effects *in vivo*, we analyzed events of early T cell polarization during primary sensitization in mice. To overcome the low frequency of naïve antigen-specific T cells in wild type mice, we established a DO11.10/BALB/c chimera using adoptive transfer, thus ensuring a defined number of $OVA_{323-339}$-specific CD4 cells in regional lymph nodes. 48 hours after intranasal instillation of $OVA_{323-339}$ alone or $OVA_{323-339}$ plus aqueous birch pollen extract (APE) to DO11.10/BALB/c chimeras, cells were obtained from draining lymph nodes, restimulated with peptide *in vitro* and on day 6 intracellular IL-4 and IFN-γ were analyzed by flow cytometry. Transfer of $5x10^6$ T cells led to 1-2% of $OVA_{323-339}$ TCR transgenic T Cells in regional lymph nodes. 48 hours after intranasal instillation of $OVA_{323-339}$, proliferation of antigen specific T cells was detected in draining, but not in non-draining lymph nodes. In comparison to exposure with $OVA_{323-339}$ alone, intranasal instillation of $OVA_{323-339}$ plus APE lead to an increase in IL-4 - and a decrease of IFN-^{3} producing antigen specific T cells, as determined after peptide restimulation. In conclusion, PALMs appear to exert immunomodulatory activities on the early phase of primary sensitization *in vivo*, that result in a bias towards Th2 polarization.

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Positive and Negative Regulation of Mast Cell Activation by Lyn via the FcεRI

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Aggregation of the high-affinity receptor for IgE (FcεRI) induces activation of mast cells. Here we show that, upon 'low-intensity' stimulation of FcεRI with monomeric IgE, IgE+anti-IgE or IgE+low Ag, Lyn (the most prominent Src family kinase expressed in mast cells) positively regulates degranulation, cytokine production and survival, whereas Lyn works as a negative regulator upon 'high-intensity' stimulation with IgE+high Ag. 'Low-intensity' stimulation suppressed Lyn kinase activity and its association with FcεRI β subunit whereas 'high-intensity' stimulation enhanced Lyn activity and its association with FcεRI β. The latter induced much higher levels of FcεRI β phosphorylation and Syk activity than the former. Downstream positive signaling

molecules such as Akt and p38 were positively and negatively regulated by Lyn upon 'low-intensity' and 'high-intensity' stimulations, respectively. On the other hand, negative regulators SHIP and SHP-1 interacted with FcεRI β and their phosphorylation was controlled by Lyn. Therefore, we conclude that Lyn-mediated positive vs. negative regulation depends on the intensity of stimuli. Studies on mutant FcεRI β showed that FcεRI β subunit-ITAM (immunoreceptor tyrosine-based activation motif) regulates degranulation and cytokine production positively and negatively depending on the intensity of FcεRI stimulation. Furthermore, Lyn-mediated negative regulation was shown to be exerted via the FcεRI β-ITAM.

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Airway and bone marrow eosinopoiesis after airway allergen exposure; role of Eotaxin-2

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Allergen-induced eosinophilic inflammation may results from the recruitment of these cells from the bone marrow (BM) via the blood, but also through local airway proliferation and final maturation. It has been shown that eosinophil CD34+ progenitors traffic to airways during allergen exposure, but the mechanisms of this traffic and the end result of the airway CD34-cells is less established.

General methodology: Mice were sensitized (OVA) and subsequently exposed to allergen repeatedly. Newly produced cells were labelled with a thymidine analog (BrdU).

Results: Adoptive transfer of of BrdU-labelled bone marrow cells (i.v.) from allergen-exposed mice to another sensitized and allergen exposed mouse, resulted in approximately 50% of BAL eosinophils being BrdU-positive, proving that these cells came from the donor bone marrow. During repeated airway allergen exposure, the number of CD34-cells in airways increase substantially, providing evidence of primitive myeloid progenitors being present in airways. FACS analysis showed that lung CD34 cells undergoing cell cycle (in S-phase) increase during allergen exposure ($CCR3^+/BrdU^+/7\text{-}AAD^+$). Culture *in vitro* of lung or BAL CD34-cells in the presence of IL-5 and/or Eotaxin-2 resulted in Eosinophil Colony Formation in the airways after airway allergen exposure. Mice treated with anti-Eotaxin-2 antibody or a CCR3 receptor antibody have a strongly reduced traffic of CD34-cells to airways. CD34-cells from both BM and blood have the capacity to release substantial amounts of IL-5 upon stimulation in vitro, and the extent of this release may be similar to that of CD3-cells in this model.

Conclusion: We conclude that the extent of airway eosinophilia in sensitized and allergen exposed mice depend to a large extent on traffic of newly produced eosinophils from the bone marrow, but also that local airway proliferation and maturation of eosinophils from primitive cells contribute. The traffic of CD34+ eosinophil progenitors to airways, and their local airway proliferation, is partly dependent on eotaxin-2 and CCR3 receptor activation.

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Is Human IgE+ Equivalent to Mouse Highly Cytokinergic IgE?

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Rationale: We have previously defined IgE+ as the IgE on basophils from a subset of highly allergic, asthmatic subjects that release histamine after stimulation with histamine releasing factor (HRF), IL-3 and D_2O. Since there have been reports of mouse monomeric IgE (termed Highly Cytokinergic {HC} IgE) causing mediator and cytokine release and phosphorylation (PY) events in rodent mast cells in the absence of antigen, we investigated if human IgE+ was HC IgE.

Methods: IgE+ was defined as causing $\geq$ 10% histamine release (HR) to HRF after passive sensitization of dextran sedimented basophils. By definition IgE- did not support HR to HRF. Once defined, serum and various purified IgEs were used to stimulate highly purified basophils generated by double percoll gradients and negative selection and examined for ERK PY by Western blots and cytokine production by ELISA.

Results: 13 myeloma IgEs, 1 commercial IgE (Calbiochem) and gp120 IgE (Tanox) , all of which are IgE-, showed no ERK PY despite using 5 ug/ml. Serum from IgE+ individuals (5 ug/ml, n=2) and IgE- subjects (5 ug/ml, n=1) did show ERK PY, with more PY seen with IgE- serum. To further clarify this, IgE+ and IgE- were purified from the serum and demonstrated minimal ERK PY (5 ug/ml, n=2 respectively). The IgE in serum from 1 IgE+ and 1 IgE- donor caused IL-13 production from basophils.

Conclusion: We have demonstrated ERK PY from 1 purified IgE+ and 1 purified IgE- subject. These IgE molecules must be further purified to assure they are monomeric. Thus, we conclude that IgE+ does not fully explain HC IgE.

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Protective effect of corm -3, a water soluble carbon monoxide releasing molecule, in a model of vascular inflammation.

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The enzyme heme oxygenase (HO) is able to split the tetrapyrrole heme ring into biliverdin, free ferrous iron, and carbon monoxide (CO). Previous reports from our group showed that exogenous CO or water-unsoluble CO-releasing molecules were able to mimic the anti-allergic and anti-anaphylactic effects of HO in isolated guinea pig hearts, in guinea pig mast cells and in human basophils, mainly through the activation of the soluble guanylyl cyclase/cGMP system. Here we report the effects of a novel, water soluble CO-releasing molecule (CORM-3) in an *in vitro* model of vascular inflammation. The effects of CORM-3 were evaluated in a coincubation of rat coronary endothelial cells (ECs) with human neutrophils (PMN), activated with the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP, 10^{-8}M), through the flow cytomeytric evaluation of cellular surface markers (CD54 on ECs and CD11b on PMN), expressed as relative fluorescence units (RFU).

The expression of CD54 upon ECs was increased after the incubation with PMN stimulated by fMLP (basal 1.3±0.2 RFU vs fMLP 3.3±0.7 RFU; $p<0.01$). CORM-3 (100 nM, 1 uM, 10 uM) was able to reduce the increased expression of CD54 from ECs (to 1.9±0.1 RFU; 2.1±0.2 RFU; 1.7±0.3 RFU; $p<0.01$), while the inactivated form of the drug (iCORM; 10uM), unable to release CO, was ineffective (2.8±0.2 RFU). Superoxide anion may also play a role in endothelium activation, since the treatment of ECs with SOD (300 IU/ml) mimicked the effects of CORM-3 upon CD54 expression (SOD 2.2±0.4 RFU vs fMLP alone 3.3±0.7 RFU). Finally, CORM-3 (100 nM, 1 uM, 10 uM) also reduced the activation of human PMN, assessed as the membrane expression of CD11b (fMLP alone 4.0±1.5 vs CORM-3 2.8±1.1 RFU; 1.5±1.4 RFU; 1.2±0.8 RFU; $p<0.01$) while iCORM and SOD were ineffective. CORM-3 was found able to induce the production of cGMP in ECs (0.76 ± 0.1 fmol/mg protein vs 1.73 ± 0.2 fmol/mg protein).

In conclusion, CORM-3 was highly effective in the reduction of PMN-induced CD54 expression upon ECs. The effect was mediated by the release of CO, since iCORM was completely ineffective. We can also suggest the involvement of superoxide anion, and of a cGMP dependent intracellular pathway.

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Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites

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While probing host skin to search for blood vessels, female *Anopheles* mosquito delivers *Plasmodium* parasites in the presence of saliva. Saliva which contains several pharmacologically active components is believed to facilitate blood feeding as well as parasite transmission to the host. Recently, we found that mosquito saliva has the capacity to activate dermal mast cells and to induce local inflammatory cell influx. Put together, these findings led us to hypothesize that mosquito saliva may facilitate parasite transmission by down-regulating the immune response through mast cell activation. Our main objective in the present work is to investigate whether saliva, through mosquito bites, controls the magnitude of antigen-specific immune responses and whether this control is dependent on mast cell-mediated inflammatory response. Using a mast cell knockin mouse model, we found that non infectious mosquito bites consistently induced MIP-2 in

the skin and IL-10 in draining lymph nodes as measured by RT-PCR. Interestingly, when mice undergoing an ovalbumin-specific DTH response were exposed to mosquito bites at the sensitization phase, we observed a down-regulation of antigen-specific T cell responses. This reduced T cell response was dependent on mast cells and mediated by IL-10. Our results provide evidence for new mechanisms which may operate to facilitate *Plasmodium* parasite progression and development within the host by mosquito bites.

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Human Mast Cells Release Oncostatin M Specifically on Contact with Activated T cells

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Most commonly known for their role in the elicitation of IgE-mediated allergic inflammation, mast cells have been implicated in a range of other non-allergic inflammatory processes. Observations such as the close physical proximity between mast cells and T cells in inflamed tissues, and the capability of the former to release a wide range of immunomodulatory mediators and to express surface molecules important in costimulation in both adaptive and innate immunity, have led investigators to propose a functional relationship between these two cell populations. We have recently demonstrated that mast cells can be activated by heterotypic adhesion to activated T cells. In an effort to systematically examine the cytokine genes that are upregulated on activation of mast cells, we used microarray analysis to perform gene expression porofiling on human mast cells activated by either IgE-cross-linking or by T cells. We further characterized one of the cytokines, which was specifically upregulated upon T cell-induced mast cell activation, oncostatin M (OSM). Expression of OSM was validated by RT-PCR and the released protein was measured by ELISA in both the LAD2 human mast cell line and in cord blood-derived human mast cells. OSM was found to be expressed and released specifically on T cell-induced mast cell activation but not on IgE cross-linking. OSM was localized to the cytoplasm as assessed by immunofluorescence and its production inhibited by dexamethasone. Mast cell derived OSM was also found to be biologically active in inducing lung fibroblast proliferation that was partially but significantly inhibited by anti OSM mAb. To the best of our knowledge, this is the first report of OSM in human mast cells. Based on the previously reported biological effects of OSM, our results suggest that production of OSM may represent one link between T cell-induced mast cell activation and the development of a spectrum of structural changes in T cell-mediated inflammatory processes in which mast cells have been found to be involved.

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Identification of nitric oxide regulated genes with potential roles in signaling in mast cells

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Exogenous nitric oxide (NO) inhibits mast cell cytokine production as well as specific consequences of allergic inflammation, and may therefore represent a critical disease-limiting mechanism. The nature of this suppressive effect, in that it is time-dependent, reversible and non-toxic, suggests that NO may regulate the expression of genes critical to cytokine production. Mast cell signaling studies show that NO inhibits Ag-induced synthesis of Fos and Jun proteins (components of the AP-1 transcription factor) and their subsequent DNA binding activity, without influencing early protein phosphorylation events. Therefore, our experimental hypothesis is that NO targets a gene (or genes) that influence(s) Fos and Jun synthesis and subsequent cytokine production. To identify NO-regulated genes we conducted gene microarray studies using a customized in-house cDNA oligonucleotide spotted array platform on which approximately 15,000 selected genes are represented. Mouse bone marrow-derived mast cells (BMMC) were exposed to S-nitrosoglutathione (a natural storage form of NO), spermine–NO (a synthetic NO source) or to control medium under optimized conditions, before extraction of RNA, reverse transcription, cDNA labeling and competitive hybridization to the array. Results were analyzed statistically by MicroArray DataBase (mAdb, NIAID) and Significance Analysis of Microarrays (SAM). At the appropriate significance level the expression of 42 genes was up-regulated >2-fold by both sources of NO. Of particular note, NO elevated expression of the gene encoding dual specificity phosphatase 1 (Dusp1) 17-fold and that encoding heme oxygenase 1 (Hmox1) 13-fold. Dusp1 (MAPK phosphatase 1) is known to specifically inactivate Erk1/2 and JNK, kinases involved in cytokine production, while Hmox1 generates bilirubin and carbon monoxide that have potential cytokine suppressive actions.

Western blot studies showed that NO elevated protein levels of both Dusp1 and Hmox1 in BMMC. In conclusion, gene microarray studies reveal several potential gene targets for the regulatory action of NO on signaling and cytokine production in mast cells. Certain of these genes and their protein/enzyme products, notably Dusp1 and Hmox1, are prime candidates as suppressive factors of mast cell cytokine expression and may therefore be implicated in NO resolution of allergic diseases.

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Regulation of human mast cell survival after aggregation of FcγRI or FcεRI

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The high affinity receptors for IgE and IgG are both known to be expressed on human mast cells. Activation of mast cells through either the IgE- or IgG-receptors leads to production and release of inflammatory mediators. One of the biologic characteristics of mast cells in allergic pathology is that these cells have the capacity to recover and regranulate after IgE-receptor mediated activation. We have previously demonstrated that the antiapoptotic protein A1, a member of the Bcl-2 family, is required for mouse mast cells to survive IgE-mediated activation (Xiang et al. J Exp Med 2001). In the present study we have investigated whether human mast cells also show similar activation-induced survival after aggregation of the IgE-receptor. Furthermore, we also measured the effect of IgG-receptor aggregation on human mast cell survival and regulation of Bcl-2 family members. Human mast cells were derived by culturing umbilical cord blood cells in the presence of stem cell factor and IL-6 until a homogenous population of tryptase positive cells was obtained. The cells were activated by IgE + anti-IgE or IgG + anti-IgG. We then measured release of beta-hexosaminidase, cell survival and mRNA expression of bcl-2 family members.

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Role of the high-affinity IgE receptor (FcεRI) β-chain as a bifunctional signal regulator and molecular mechanisms for regulation of its gene expression.

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FcεRI is a key molecule for mast cell activation in allergic reaction, consisting of three subunits, one α, one β and a homodimer of γ subunits. The β-chain is recently focused on as a signal regulator for activation of mast cell, a conductor of allergic inflammation.

In immunoreceptor tyrosine-based activation motif (ITAM) of the β-chain, there are unusually three tyrosine residues, one non-canonical residue between the two canonical residues. We evaluated roles of each tyrosine residue for FcεRI signaling. Into the β (-/-) bone marrow derived mouse mast cells, mutated β-chains (each tyrosine residue in ITAM was replaced by phenylalanine) are introduced and these transfectants were stimulated with IgEû0Ag complex or monomeric IgE. With IgEû0Ag stimulation, the β-ITAM mutated transfectants demonstrated enhanced release of inflammatory cytokines, on the contrary their chemical mediator release was suppressed, and monomeric IgE stimulation, showed similar results.

These FcεRI-stimulated transfectants displayed enhanced activation for tyrosine kinases (ERK1/2, p38MAPK, IKKβ) and NFkB and phosphatases. On the contrary, Ca^{2+} mobilization, Lyn, SyK, PLC γ 1/2 activation were decreased.

Taken together, mast cell activation upon FcεRI engagement is finely regulated by the β-chain which works bifunctionaly, namely enhances degranulation and suppresses cytokine production. For this bifunctional regulation, the non-canonical unique tyrosine residue plays a crucial role.

On the other hand, we screened for cis-acting elements over the entire region of the human FcεRI β-chain gene and discovered multiple elements. Of which, the element in the 4th intron, 4180-4260nt, binds MZF-1 and FHL-3 complex, down regulating the β-chain gene expression.

GM-CSF, which was reported to decrease FcεRI expression, induced accumulation of FHL3 in the nucleus and enhanced expression of MZF-1.

In conclusion, the FcεRI β-chain is a critical molecule to define sensitivity and reactivity of mast cells in allergic reaction, therefore it can be a good molecular target for medical intervention of allergic inflammation.

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Control of exaggerated allergic inflammation through cleavage of IgE by effector cell-derived proteases

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Immunoglobulin E (IgE)-mediated allergies are a major health problem. Cross-linking of IgE bound to high affinity receptors (FcεRI) on mast cells releases pro-inflammatory mediators, cytokines and proteolytic enzymes and is a key event in allergic inflammation. We show that ß-tryptase, a major protease released during mast cell activation cleaves IgE and abolishes binding of IgE to allergens as well as to FcεRI. Furthermore, we demonstrate the presence of IgE degradation products in tryptase-containing tissue fluids collected from sites of allergic inflammation. The *in vivo* relevance of our experiments is demonstrated by the fact that protamine, an inhibitor of heparin-dependent proteases, prolonged IgE-mediated allergic skin reactions. We suggest that protease-mediated cleavage of IgE is a natural, hitherto unknown mechanism for controlling overwhelming allergic inflammation.

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Mechanisms of inhibition of human allergic Th2 immune responses by regulatory T cells induced by interleukin 10-treated dendritic cells and transforming growth factor beta.

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In grass pollen allergic individuals T cell anergy can be induced by IL-10-treated dendritic cells (IL-10-DC) resulting in a decreased proliferation and Th1 as well as Th2 cytokine production. This study was set out to analyze whether such anergic T cells are able to suppress the function of other T cells and to analyze the role of TGF-ß as potential inducer of regulatory T cells (Treg) in the periphery in this system. Freshly isolated $CD4^+$ or $CD4^+CD25^-$ T cells from grass pollen allergic donors were stimulated with autologous mature monocyte-derived allergen-pulsed dendritic cells in the presence or absence of T cells previously cultured with IL-10-DC- and/or TGF-ß. Anergic T cells induced by allergen-pulsed IL-10-treated DC or allergen-pulsed DC and TGF-ß alone enhanced IL-10 production and strongly inhibited IFN-gamma production of fresh peripheral $CD4^+$ or $CD4^+CD25^-$ T cells while proliferation and Th2 cytokine production were only slightly reduced. The addition of TGF-ß or the use of allergen-pulsed IL-10-treated DC and TGF-ß had an additional effect leading to a much stronger suppression of Th2 cytokine production and proliferation. Suppression was not antigen-specific and was mainly mediated by cell-to-cell contact and by the molecule programmed death-1 (PD-1) and only partially by CTLA-4, TGF-ß and IL-10. These data demonstrate that regulatory/suppressor T cells that also suppress Th2 cytokine production are induced most efficiently by DC that had been pretreated with IL-10 and by TGF-ß. This might be exploited for future therapeutic strategies for allergic diseases.

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Extracellular Traps: A New Mechanism Used by Eosinophils to Fight Against Bacteria

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Background & Aims: Although eosinophils are considered as being useful in defense mechanisms against parasites, their exact function(s) in innate immunity remains unclear. The aim of this study was to better understand the role of eosinophils infiltrating the gastrointestinal tract. *Methods:* We analyzed freshly purified blood eosinophils in vitro as well as biopsies using immunofluorescence and confocal microscopy to characterize intracellular components of eosinophils that are released during the course of a bacterial infection of the intestine. *Results:* We demonstrate that activated eosinophils release DNA in conjunction with granule proteins and that both together form extracellular traps able to bind and kill bacteria in vitro. Eosinophil extracellular traps were identified in vivo in the course of bacterial infection of the intestine. *Conclusion:* Eosinophils contribute to innate immune responses by fighting against bacteria in the extracellular space.

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Assembly, dynamics and activity of human membrane IgE

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Human membrane IgE (hmIgE) constitutes the B cell receptor (εBCR) of B cells responsible for IgE production. Given the complex genetic and environmental factors, B cells exposing εBCRs ultimately determine IgE-related immune-disregulations, such as allergic diseases and hyper IgE syndromes. Concerning the IgE-expressing B cells, the differentiation stages leading to plasmacells and production of secretory IgE are yet debated and unclear. Due to their exceedingly low number, εBCR^+ plasmacell precursors and memory B cells have been highly elusive to date. Allegedly, the expression level of membrane IgE on the surface of B cells could be lower than that of other Ig isotypes or εBCR^+ cell lifespan shorter, envisaging an intrinsic instability either at the genetic or at the protein level. In an attempt to understand the molecular features of hmIgE at the protein level, an extensive characterization of the assembly, transport, membrane dynamics and ability to bind different ligands has been carried out.

The two isoforms (long and short) of hmIgE and a truncated version of hmIgE long (deprived of the portion N-terminal to the Cε3 domain) have been transfected in a variety of mouse B cell lines (WEHI, A20, J558L and sp2/0), representing various B cell differentiation stages. In A20 cells, hmIgE long and short assemble to form competent εBCRs. In J558L and sp2/0, hmIgE long and its truncated version, respectively, were exported to the cell surface even in absence of the BCR accessory Igα-Igβ heterodimers. Interestingly, all hmIgE variants were capable to bind soluble human FcεRIα and to activate (in an antigen independent manner) human FcεRI expressed on the surface of transfected RBL cells. Assembly of hmIgE was further characterized in terms of endoplasmic reticulum-resident glycosylation and heavy-light chain association intermediates. Finally, the rate and dynamics of hmIgE association to membrane raft microdomains has been characterized, revealing a complex aggregation-dependent pattern.

In conclusion, hmIgE is seemingly acting as a fully-equipped BCR and exerts a previously undescribed binding activity *via* its Fc portion.

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Caspase activation and loss of mitochondrial membrane potential precedes phosphatidylserine exposure in CD45-dependent eosinophil apoptosis

Morgan Blaylock, Garry Walsh

Background

There is much interest in the intracellular mechanisms governing eosinophil apoptosis. We previously demonstrated that eosinophils express CD45 isoforms in a cell-type specific fashion (Blaylock et al. *Clin. Exp. Allergy* 2003;33:936) and that treatment of eosinophils with CD45 mAb elevates their constitutive apoptosis (Blaylock et al. J Allergy Clin Immunol. 1999;104:1244). Caspases are key molecules in the control of apoptosis but their contribution to eosinophil apoptosis is poorly understood. We therefore examined the role of caspase-3, -8 and -9 in eosinophils ligated with CD45 mAb in parallel with externalization of phosphatidylserine and changes in mitochondrial transmembrane potential ($\Delta\Psi_m$).

Methods

Purified human eosinophils were cultured for 2, 4 and 8 hours with optimal concentrations of CD45RA, CD45RB, CD45RO, isotype-matched control mAb or dexamethasone (10^{-6}M). Caspase activity was analyzed using fluorochrome inhibitor of caspases technology in parallel with phosphatidylserine externalisation measured by Annexin V binding. Both parameters and changes in ($\Delta\Psi_m$)were analysed using flow cytometry.

Results
Eosinophils treated with CD45RA or CD45RB mAb did not exhibit significant increases in phosphatidylserine exposure at up to 8 hour post-ligation. The same cells exhibited significant ($p<0.05$) caspase-3 activation at 4 and 8 hours post-treatment with CD45RB mAb. Significant ($p<0.05$) caspase-8 activation followed CD45RB treatment at 2, 4 and 8 hours while CD45RA failed to elicit a significant response. Caspase-9 activation was evident at all time points studied following CD45RB ligation while CD45RA activated caspase-9 at 8 hours. CD45RO ligation or dexamethasone treatment failed to activate caspases above constitutive levels at these early time-points although significant apoptosis was observed in dexamethasone-treated eosinophils after 24 hours of treatment. CD45RB-dependent caspase activation was associated with changes in ($\Delta\Psi_m$) at 8 hours post-treatment; the same loss of ($\Delta\psi_m$) was not seen with CD45RA.

Conclusions
These observations suggest that different caspase pathways are involved in the apoptosis-inducing events following eosinophil CD45-dependent receptor-ligation and these precede membrane phosphatidylserine exposure. In contrast, dexamethasone did not activate caspases at these early time-points. These findings emphasise the early nature of the intracellular events controlling mAb-dependent apoptosis induction in human eosinophils and may aid development of more targeted and effective asthma therapy.

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Gene Expression in the middle ear of mast cell deficient mice

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We have previously shown that mast cells contribute to the inflammatory response to non-typeable Haemophilus influenzae (NTHi) in the middle ear (ME) of mice. To evaluate the mechanisms by which mast cells may contribute to ME inflammation, we assessed the global gene expression in the ME of mast cell deficient W/Wv (MCD) mice and wild-type congenic mice. The middle ear mucosa was surgically removed from groups of MCD and wild-type congenic control mice prior to, and 3 and 6 hours after, inoculation of NTHi into the middle ear cavity. An Affymetrix mouse genome array containing more than 45,000 genes was used. Expression was analyzed using variance-modeled posterior inference. Prior to bacterial inoculation, 27 genes were down-regulated in MCD mice, including, as expected, mast-cell specific granular proteases. An additional 245 genes were upregulated in these mice. NTHi instillation into the ME of wild-type control mice induced 513 genes (3h) and 658 genes (6h) respectively when compared to saline injected controls at these same time periods, while 2 and 188 genes were down-regulated. Compared to congenic wild-type mice, 90 genes were down-regulated in the MCD mouse ME at 3h after NTHi instillation with mast cell proteases being the most down-regulated. Other genes with expected roles in inflammation including genes related to IL-1, interferon and chemokines, were also expressed at significantly lower levels in the MCD ME at this time. Another 125 genes were up-regulated in MCD mice at 3h. At 6 h, although 25 genes were down-regulated in MCD ME, none of the inflammatory genes suppressed at 3h remained suppressed. A total of 199 genes were up-regulated at 6h including several receptors for inflammatory mediators. These results suggest that mast cells are important contributors to acute inflammation during bacterial otitis media, and the IL-1 and interferon may well be central to this role.

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Naïve CD4⁺ T Cell Activation by Antigen-Presenting Airway Eosinophils

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In addition to the conventional "effector" functions of eosinophils, evidence that eosinophils function as antigen-presenting cells (APCs) has been increasing. A major distinction amongst potential APC types is between amateur and professional APCs. Amateur APCs stimulate only previously activated T cells and T cell hybridomas, whereas professional APCs are capable of initiating T cell responses. To investigate whether eosinophils are capable of initiating T cell responses in vivo, eosinophils were isolated from the spleens of IL-5 transgenic BALB/c mice by Percoll followed by MACS, and contamination with other APCs including dendritic cells was excluded. Co-culture of eosinophils with GM-CSF increased their expression of costimulatory molecules including MHC-II. The GM-CSF-stimulated eosinophils were allowed to take up OVA antigen in vitro and then intratracheally injected into wild-type BALB/c mice that had received intravenous infusions of Ag-specific CD4⁺ T cells from DO11.10 OVA TCR transgenic BALB/c mice 24h earlier. By alternatively using GFP-labeled eosinophils from IL-5 & GFP double transgenic mice and fluorescently conjugated OVA–beads, we demonstrated by fluorescence microscopy that the Ag-loaded eosinophil APCs were physically interacting with naive OVA TCR CD4⁺ T cells in the draining paratracheal lymph nodes (PLNs) 24h after eosinophil transfer, while Ag-free eosinophils were randomly distributed across the PLNs with the donor CD4⁺ T cells. The physical interaction between Ag-loaded eosinophils and Ag-specific CD4⁺ T cells resulted in activation and proliferation of the naïve Ag-specific CD4, as evaluated by an early T cell activation marker CD69 and BrDU incorporation, respectively. However, this eosinophil APC function was almost completely impaired if eosinophils were pre-treated with RBC lysis buffer containing ammonium chloride, which inhibits antigen processing by eosinophils. Our data indicate that eosinophils may function as professional APCs to initiate T cell responses to a given antigen.

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Baseline and stimulated turnover of cell surface c-Kit expression in different types of human mast cells

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Background: The receptor tyrosine kinase c-Kit is of fundamental relevance to mast cell (MC) development and maintenance. Kit expression seems tightly regulated at different levels, including transcription, shedding, and internalization. Nothing is known about the regulation of Kit cell surface levels in normal tissue-derived human MC, however. Likewise, the baseline turnover of Kit has not been addressed yet.

Methods: Terminally differentiated MC were isolated from human skin, according to established protocols. The less mature MC lines LAD2 and HMC-1 were used for comparison. Cells were exposed to different treatments including stem cell factor (+/-STI571), cycloheximide, actinomycin D, and combinations thereof for times ranging from 20 min to 16 h. Expression levels of Kit and other cell surface receptors were determined by flow-cytometry.

Results: Ligand-induced Kit internalization was a universal mechanism and detectable in HMC-1, LAD2 and skin MC. STI571, an inhibitor of the intrinsic Kit kinase, was able to significantly delay Kit internalization in LAD2 but also in HMC-1 cells that carry the D816V mutation and had been considered unresponsive to STI571. STI571 alone, though suppressing SCF-driven internalization, unexpectedly induced downregulation of Kit on its own. Investigations into the natural turnover of Kit expression in the three types of MC revealed that Kit is rapidly affected by the inhibition of fundamental cellular processes, even in non-proliferating skin MC. Only a minor decrease of other cell surface receptors (ICAM-1, ICAM-3, β1 integrin chain) was noticed in the same time frame. On combined treatment, cycloheximide, actinomycin D and SCF displayed additive effects, resulting in an almost complete disappearance of Kit from the cell surface.

Conclusions: Kit represents a rapidly cycling cell surface receptor. Not only is it rapidly internalized upon binding of its ligand, but it is also heavily affected by inhibition of de-novo-protein synthesis or transcription when viewed against the background of other receptors. The relatively short half-life of Kit even in the absence of ligand suggests that carefully balanced Kit levels are of fundamental importance to MC. In addition, it is shown that STI571 can display effects in cells expressing one gene copy of kit that carries the D816V substitution.

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Purification of the natural peanut allergen Ara h 1

Wolf-Meinhard Becker

Purification of immunological pure Ara h 1 from peanut extract is not trivial, since Ara h 1 consists of several isoforms. Published methods using a three step technique to separate the extract at first by anion exchange chromatography (AIEC), followed by an affinity chromatography step on immobilized ConA using the glycosylation of Ara h 1 as a principle of separation. As a third step size exclusion chromatography (SEC) is applied. However, it is not able to prevent Ara h 1 from being contaminated by Ara h 3/Ara h 4. The problem is that Ara h 1 and Ara h 3 /Arah 4 , which are isoforms , build under natural conditions a big complex of roughly 200 kDa, of which Ara h 3/Arah 4 are the main components. All column chromatographic methods interact which such a complex which may be the reason for the difficulties to circumvent contaminations with Ara h 3 /Ara h 4.

Therefore, we tested the extraction efficacy of Ara h 1 and Ara h3/Arah 4 in dependence on the pH of the extraction buffer and found, that Ara h 1 is extractable at a pH 5.0 from peanut flour, whereas under these conditions Ara h3/Ara h 4 are nearly insoluble. This peanut extract was the source material for starting Ara h 1 purification. At first the extract was separated on immobilized ConA. The alpha-methyl-mannoside eluate was analysed by Western blotting using Ara h 1, Ara h 3/Ara h 4 and Ara h 2 specific monoclonal antibodies and sera of peanut allergic patients. The preparation contained Ara h 1 as main component and was free from Ara h 3/Ara h 4 but still contaminated by probably break down products of Ara h 1 at 31 kDa. A further separation step on Superdex75 (SEC) led to a depletion of this fragment. This purification strategy omitting an AIEC step creates the preparation for authentic natural Ara h 1.

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Apples can drive birch pollen-allergic patients nuts! Immunological cross-reactivity as basis for food allergy

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Birch pollen-allergic patients frequently develop allergic reactions to certain foods, e.g. apples, hazelnuts and celery. These aliments contain proteins which possess a high homology with the major birch pollen allergen, Bet v 1. Consequently, Bet v 1-specific IgE antibodies cross-react with these allergens resulting in IgE-mediated allergic symptoms, e.g. the oral allergy syndrome after consumption of fresh foods. We have focussed on the T cell cross-reactivity between Bet v 1 and the related food allergens Mal d 1 (apple), Cor a 1 (hazelnut) and Api g 1 (celery). We found that these Bet v 1-related food allergens contain T cell epitopes matching relevant T cell-activating regions of Bet v 1. Furthermore, we showed that the food allergens induced proliferation and cytokine production of Bet v 1-specific T cells. In particular, the highly conserved immunodominant T cell epitope, Bet v $1_{142-156}$, was involved in this cellular cross-reactivity. In general, Bet v 1-related food allergens undergo rapid gastric degradation leading to the loss of their IgE-binding capacity. We speculated that T cell epitopes, which are short linear peptides, may survive gastric as well as pancreatic digestion. To simulate gastrointestinal digestion, recombinant Mal d 1, Cor a 1 and Api g 1 were incubated with pepsin followed by trypsin. The digested allergens were analysed for their ability to bind IgE, to induce basophil activation and to stimulate T lymphocytes. Whereas the gastrointestinal digested allergens neither bound IgE nor induced mediator release, proteolytic fragments of Mal d 1 and Cor a 1 still induced proliferation and cytokine synthesis in Bet v 1-specific T cell lines and clones. Using mass spectrometry the proteolytic fragments were sequenced and several peptides matching T cell epitopes of Bet v 1 were identified. In summary, gastrointestinal degradation of Bet v 1-related food allergens eliminates their potency to induce IgE-mediated reactions but does not completely abolish their potency to activate Bet v 1-specific T lymphocytes. Our data emphasize that birch pollen-related foods represent potent stimuli of pollen-specific T cells which may be relevant for perennial stimulation of pollen-specific T cells in patients with a seasonal allergy.

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Mass spectrometric analysis of natural and recombinant preparations of pollen (birch, grass, olive) and house dust mite allergens

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Traditionally, diagnosis and immunotherapy of allergic diseases are based on the use of aqueous protein extracts of natural allergen sources. But these extracts are heterogeneous and these mixtures of allergenic as well as non-allergenic components are difficult to standardize. One of the aims of the European Union CREATE project is to analyze natural and recombinant major allergens from birch pollen (Bet v 1), grass pollen (Phl p 1, Phl p 5), olive pollen (Ole e 1) and house dust mite (Der f 1, Der f 2, Der p 1, Der p 2) regarding their physico-chemical and immunological properties. On the basis of this and other analysis, allergen preparations will be identified that meet the requirements to become certified reference material for allergenic products. Here, we present the mass spectrometric investigation of the recombinant allergens and their natural counterparts from the create project. Tryptic peptides of the natural and recombinant allergens were separated by Capillary HPLC and analyzed by ESI-MS/MS (ElectroSpray tandem Mass Spectrometry) using a Q-TOF (Quadrupole-Time-of-flight) mass analyzer. Peptide mass and sequence were established from MS data and MS/MS fragmentation patterns of the peptide. Analysis of intact proteins was performed by direct infusion into the ESI-QTOF mass spectrometer. The mass spectrometric analysis contributed to the confirmation of identity (sequence) of the allergen preparations and to the investigation of their purity and homogeneity (isoform composition). In addition, chemical and post-translational modifications such as carbamylation, deamidation of asparagines, oxidation of methionines, and protein glycosylation of recombinant and/or natural preparations were detected. By sequencing tryptic peptides from purified natural allergens, it was also possible to identify at the protein level new isoforms of Bet v 1, Phl p 1, Phl p 5, and Der p 1.

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Evidence for high Bet v 1 concentrations in ambient air in another compartment than birch pollen?

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We monitored the daily concentration of the major birch pollen allergen Bet v 1 in ambient air by high-volume sampling and separation into PM_{10}, $PM_{2.5}$ and $PM_{0.12}$ during the birch pollen season 2005 in Munich, Germany.

Methods: 2 Chemvol® high-volume cascade impactors sampling PM_{10}, $PM_{2.5}$ and $PM_{0.12}$ were run in parallel, 10m apart at 900 and 1500l/min, 1.80m over ground. One PM_{10} stage, run at 1500l/min was greased. In addition a Burkard pollen trap was installed. Filters were extracted with 0.1M NH_4HCO_3 pH8.0, lyophilized and Bet v 1 was determined with a Bet v 1 specific sandwich ELISA. Pollen in the different PM fractions was counted by scanning electron microscopy.

Results: Bet v 1 could be monitored reliably in ambient air. Pollen was found in the PM_{10} and $PM_{2.5}$ fractions. Greasing PM_{10} did not completely stop birch pollen from entering $PM_{2.5}$. On some days we found over 50% more Bet v 1 in $PM_{2.5}$ than could be explained by the presence of birch pollen.
We also found 10 consecutive days after the major birch pollen flight with less than 10% peak pollen concentration in the air, but Bet v 1 levels mainly in the PM_{10} fraction at 60% of those at peak pollen flight.

Conclusions: Respirable particles smaller than pollen containing Bet v 1 can be found in the air during birch pollen flight. Furthermore, high levels of Bet v 1 in ambient air were found not corresponding with pollen flight in periods following pollen peaks. Thus another Bet v 1 containing compartment seems to exist in ambient air, besides the pollen compartment.

Concentration of the major birch allergen Bet v 1 in ambient air does not correlate with birch pollen counts.

Calculations:
Sum total in 10 consecutive days about 60% of days max:

Sum 900l/min corrected to 1000l/min Bet v 1 pg/m3 20/4-5/1 without 26/4 and 27/4= 233,7+/- 28.06

Peak Bet v 1 on 15/4 (not 16/4)= 380 pg Bet v1 /m3 (corrected on 1000l/min)

233,7/380= 61,5%

if explained by more potent pollen then pollen must be 6.6 times more potent

bet v 1/pollen 14/4-16/4= 0,205+/-0.099

bet v 1/pollen 20/4-1/5 without 25/4-26/4-27/4= 1.34+/- 0.438

1.34/0.205= 6.5 times more potent. On 25/4 a lot of bet v 1 but no pollen so I think not true that super potent pollen explain difference

Small particles:
In a period after peak birch pollen flight, with less than 10% of pollen in the air compared to peak pollen count, there were still 60% of the Bet v 1 concentrationsmainly in the PM_{10} fraction.

After the major birch pollen flight there were 10 consecutive days where Bet v 1 concentrations were 60% of those at peak altough less than 10% of pollen compared to the peak were found, mainly in the PM_{10} fraction.

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Nitration of allergens: Another pollution effect to worry about

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Nitration of tyrosine residues contained in endogeneous proteins occurs for example in inflammation, cellular stess and ageing, and this nitration is discussed as an immune stimulus. In the environment, NO_2 and O_3 are associated with summer smog, and if these pollutants exceed certain limits, various levels of smog alarm are initiated. It was unknown that these pollutants affect proteins, until Franze et al. published in 2005 the finding that proteins get nitrated on tyrosine residues by simple exposure to urban air. Among the proteins thus identified was Bet v 1, which raises the possiblity that nitration of inhaled allergens may affect the immune response.

We have investigated the immunogenic and allergenic properties of *in vitro*-nitrated allergens. Ovalbumin and recombinant Bet v 1a were used in unmodified and in nitrated forms, where the allergens contained 3-4 residues of 3-nitrotyrosine.

Splenocytes from DO11.10 mice showed upon stimulation with nitrated vs. unmodified allergen enhanced proliferation and increased production of IL-5 and IFN-γ. Similar results were obtained using splenocytes from BALB/c mice sensitized with either Ovalbumin or Bet v 1a in unmodified or nitrated form: Proliferation and cytokine production were enhanced by using the appropriate allergens in nitrated form. Furthermore, sera of mice sensitized with nitrated allergens (ovalbumin or Bet v 1a) showed elevated levels of specific IgE, IgG_1, and $IgG_{2A,}$ compared to sera from mice sensitized with unmodified allergens. Moreover, cross-reactivity between antibodies against these otherwise unrelated allergens was observed for the nitrated forms. The effects on IFN-γ production and IgG_{2A} levels indicate that the enhancement of immune reactions is not exclusive for the allergic branch. However, allergens can of course be expected to specifically induce allergic sensitivity.

Human sera from birch pollen-allergic individuals were analysed for presence of IgE specific for nitrated Bet v 1a. We found higher amounts of specific IgE binding to nitrated compared to untreated Bet v 1a. In light of these findings, it is likely that nitration enhances allergic responses, which may contribute to an increased prevalence of allergic diseases in polluted urban environments.

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***Artemisia* and *Ambrosia* hypersensitivity: co-sensitization or co-recognition? A study using recombinant weed pollen allergens**

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Ragweed (*Ambrosia*) and mugwort (*Artemisia*) have nearly identical flowering periods. Clinical and serological studies showed that ragweed and mugwort sensitization are often associated and this poses relevant clinical problems in patients for whom specific immunotherapy is warranted. Therefore, the goal of this study was to determine whether the concomitant ragweed and mugwort pollen hypersensitivity is the result of co-sensitization or of co-recognition by using purified recombinant allergens. Sensitization to ragweed and mugwort pollen was assessed by SPT in all patients reporting allergic symptoms in August and September. IgE reactivity of sera from 42 SPT positive patients (26 Amb+/Art+, 14 Amb+/Art-, and 2 Amb-/Art+) to ragweed and mugwort pollen extract as well as to several recombinant ragweed (rAmb a 1, rAmb a 5, rAmb a 6, rAmb a 8, rAmb a 9, Amb a 10) and mugwort (rArt v 1, rArt v 4, rArt v 5, rArt v 6, 3 EF-hand calcium binding protein) allergens was detected by dot-blot and ELISA. In-vitro, 90% of ragweed-allergic patients reacted with rAmb a 1. Reactivity to other ragweed allergens ranged between 20 and 35%. 46% of the mugwort-sensitized patients recognized rArt v 1, 25% reacted to Art v 4, Art v 5, and Art v 6, and 7% recognized the 3-EF hand calcium binding protein. Immunoblot inhibition experiments showed that pre -incubation with ragweed pollen extract did not significantly decrease IgE reactivity to mugwort allergens. Taken together, our results indicated that (i) there is very limited cross-reactivity between Amb a 1 and the homologue Art v 6 and between Art v 1 and its homologue in ragweed; (ii) the major allergens Amb a 1 and Art v 1 can thus be considered as markers for ragweed and mugwort sensitization, respectively. We concluded that patients showing both ragweed and mugwort positive SPT and/or RAST are in fact co-sensitized. Therefore, patients with severe allergic symptoms in August and September should undergo specific immunotherapy with both mugwort and ragweed pollen allergens.

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Identification of the major components of the dust mite proteome and the full repertoire of its allergens

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House dust mites are among the most important source of allergens in the indoor environment. Very little however is known about the mite proteome apart from the allergenic components that have been characterized. Knowledge of the other components may be useful in enhancing our understanding of host allergic responses, mite control, monitoring and biology. As mites are "non-genome sequenced" organisms, proteomic studies on them are a challenging task. In order to understand its underlying components, proteins from *Dermatophagoides farinae* were extracted and separated by 2D electrophoresis. Coomassie stained spots were excised and subjected to matrix-assisted laser desorption/ionization (MALDI) and tandem mass spectrometry (MS-MS). The peptide mass fingerprints (PMF) and MS-MS sequences were compared to *in silico* fingerprints generated from our in-house mite expressed sequence tag (EST) database. One hundred of the most abundant spots were identified and good MS spectra were obtained. Many of these spots appear as protein isoforms – matching the same sequence contig composing of various homologous ESTs. The group 1, 2, 5, 7, 10, 13 and 20 mite allergens were among these major contigs. Careful analysis indicated that about 32 of the top 100 proteins in the proteome were allergenic proteins. The other known allergens did not appear as abundant proteins. Bioinformatic analysis indicated that many of the major mite proteins are novel with unknown functions, while others show homology to putative gene products from the *Drosophila*, common structural, biosynthetic or metabolic proteins such as actins, cuticle like proteins, esterases, kinases, ferritin, and many more. Using the EST database, we were also able to identify more than 40 groups of antigens with high sequence similarities to allergenic components. This study demonstrates that proteome identification can still be performed on a non-genome sequenced organism using PMF and tandem MS-MS with the help of expressed sequence tags. The expressed sequence tag database was not only useful in assisting the identification of the major proteins but also indicating potentially the full repertoire of allergens present in dust mites.

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Cloning of parvalbumin isoforms from codfish (*Gadus morhua*) and frog (*Rana esculenta*)

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Background: Parvalbumin has been identified as the major highly cross-reactive fish allergen. This abundant protein present in the white muscle of many fish species has a conserved calcium binding domain (EF-hand). Cross reactivity among fish species and frog has been reported. Even cross reactivity to a human autoantigen (Hom s 4) has been published. So far most of the characterized allergens belong to the β-lineage and little is known about the α-lineage from fish and frog. The aim of the study was to clone and molecularly characterize the range of parvalbumins from codfish (*Gadus morhua*) and frog (*Rana esculenta*).

Material and Methods: RNA was isolated from codfish and frog and RT-PCR performed. PCR products were cloned into TA-cloning vector and sequenced according to established methods.

Results and Conclusions: From codfish cDNA sequences coding for β- as well as α-lineages were identified with a coding region of 330 bp length. From frog also α- and β-isoforms were isolated with 333 and 330 bp length, respectively.

By the use of defined isoform sequences it will be feasible to investigate whether these sequence differences determine the quality of IgE binding capacity as well as the range of cross reactivity.

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Critical assessment of interchangeability of cross-reactive food allergens in diagnostic tests for specific IgE

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Background: IgE antibody binding to natural food extract is affected by both the nature of the allergens present in the food and their representation in the extract. Different results obtained with extracts of related foods do therefore not necessarily imply allergen uniqueness among those foods but may instead be due to unequal allergen abundance in the extracts. Many vegetable foods contain a conserved set of cross-reactivive allergens and in the design of cost-efficient reagent panels, cases of inter-species diagnostic equivalence between similar allergens needs to be utilized in order to reduce redundancy. To support interchangeability of allergens in a prospective context, evidence beyond the demonstration of cross-reactivity for selected patients' IgE antibodies is required.

Purpose: To examine the level of interchangeability between related food allergens, with respect to both detection and quantitation of specific IgE.

Methods: Experimental ImmunoCAP tests were used to measure specific IgE antibody binding to recombinant allergens from cherry, peach, hazelnut, apple, pear, celery, carrot, peanut and soy bean in serum samples collected in different clinical studies, unselected with respect to differential IgE antibody reactivity.

Results: Measurements of IgE binding to members of three allergen families were compared in a pairwise fashion to assess global epitope similarity within each family. The allergen families and their members were PR-10: Pru av 1, Pru p 1, Cor a 1, Mal d 1, Pyr c 1, Api g 1.01, Api g 1.02, Dau c 1.3, Dau c 1.02, Ara h 8 and Gly m 4; ns-LTP: Pru av 3, Pru p 3, Cor a 8, Mal d 3 and Pyr c 3; profilin: Pru av 4, Pru p 4, Cor a 2, Mal d 4, Pyr c 4, Api g 4 and Dau c 4. High levels of IgE binding similarity was observed among the profilin and ns-LTP families while significant diversity was found within the PR-10 family. In individual pairwise comparisons, IgE dataset correlations largely mirrored scores of amino acid sequence identity and ranged from r=0.51 to r=0.99.

Conclusion: The analysis supports interchangeability of several of the allergens tested and demonstrates the unique and irreplaceable nature of others.

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Determination of the B-cell epitope of hyaluronidase (Hya), a major bee venom allergen, from the crystal structure of Hya in complex with a Fab fragment of a monoclonal antibody.

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Honeybee venom contains proteins that may induce life-threatening IgE-mediated allergic reactions in humans. The allergic reaction is initiated by the allergens cross-linking the Fc-receptor-bound IgE antibodies on the surface of mast cells leading to a release of histamine and other mediators responsible for the immediate hypersensitivity reaction. Evidently, the allergen-antibody interactions are crucial for the triggering of allergic responses and thus, the knowledge of the structures of such complexes is indispensable for the development of improved allergen specific immunotherapy (SIT). Immunotherapy with venom constituents is an effective treatment of insect allergy but is not without danger: 20-40% of patients hyposensitized with bee venom develops allergic side effects. A better tolerated but equally effective SIT is therefore required, in particular for bee venom.

This work describes the first experimentally determined B-cell epitope of hyaluronidase (Hya), a major allergen of bee venom. The epitope was inferred from the crystal structure of recombinant Hya in complex with the Fab fragment of a monoclonal murine anti-Hya IgG1 antibody, determined by X-ray diffraction. Since the mAb showed a highlyinhibitory effect on the binding of patients' sera IgEs to Hya, the Hya-IgG interaction surface provides an indirect information on Hya interaction with IgE. The Hya epitope in contact with Fab is a linear array of nine amino acids which fit tightly into the deep pocket formed by the six complimentary determining regions (CDRs) of the Fab domain. The epitope (888 $Å^2$) is located at the tip of an helix-loop-helix motive of Hya, which resembles a 'handle' that protrudes away from the globular molecule. All nine residues are involved in the interaction with Fab: the polar and charged residues are located at the periphery of the Hya binding surface whereas the two hydrophobic residues from the loop region are fully buried in the Hya/Fab interface. The total number of interactions between Hya and Fab include 12 Van der Waals contacts, 8 Hydrogen bonds and 5 salt bridges. Two approaches are planned as a step towards a development of an improved SIT namely, (i) production of the hypoallergenic Hya variants by site directed mutagenesis and (ii) the synthesis of B-cell epitope derived linear peptide(s).

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Recombinant hybrid molecule consisting of rPhl p 1, 2, 5 and 6 : is it the best combination for grass pollen diagnonis in a group of French patients?

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Background : In a previous study we have shown that over 50% of grass allergic patients recognized Phl p 1, 2, 4 and 5. We also demonstrated by skin prick testing the in vivo activity of recombinant hybrid molecule (HM) consisting of rPhl p 1, 2, 5 and 6 in a group of French patients. We compared in the same population the detection of specific IgE against a natural grass pollen extract, this hybrid molecule and a battery of recombinant allergens.

Methods: Thirty five French grass pollen allergic patients were tested by ELISA for the presence of specific IgE against the natural crude grass pollen extract (9 species, Allergon), the hybrid molecule and rPhl p 1, 2, 5, 6, 7, 12, 13 and nPhl p 4. Specific IgE levels were considered positive at a level superior to 0.1.

Results: We found a significant correlation between HM specific IgE and natural grass pollen extract IgE ($r = 0.972$, $p<0.001$). More patients were positive with the HM (97.1% against 88,6%), with a high geometric mean of specific IgE levels (1.27 against 0,91). IgE binding frequency against rPhl p 1, 2 and 5 roughly corresponded to those reported earlier. The frequency of IgE against nPhl p 4 was 77%, while the rPhl p 6 binding frequency of sensitization was 65.7%. The 3 highest specific IgE levels were observed for rPhl p 1, 5 and 4.

Conclusions: Both in vivo and in vitro studies confirm that the HM is an accurate tool for diagnosis.

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Epitope mapping and characterization of the binding specificity of monoclonal antibodies directed against allergens of grass group 1

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Allergens of grass group 1 are very potent IgE-binding components with molecular masses of 30 - 35 kDa which are found in all grass species. For determination of group 1 in allergen extracts as well as a diagnostic marker we examined three monoclonal antibodies HB7, IG12 and Bo14 in detail. They had been raised against timothy grass pollen *(Phleum pratense)* and were successfully used to identify Phl p 1 by Western blotting as well as by ELISA under native conditions. Thus, the epitopes are accessible and should be located on the allergen surface. Employing inhibition tests we were able to demonstrate that neither of the monoclonal antibodies bound to IgE-reactive epitopes nor competed among each other for binding sites.

To determine the epitopes of the three monoclonal antibodies we performed a screening with recombinant Phl p 1 fragments. Further

studies were done by the PEPSCAN and SPOT techniques using overlapping synthetic decapeptides with an increment of two amino acids covering the complete Phl p 1 molecule. The monoclonal antibodies bound to epitopes located on different regions of the allergen. For HB 7 we could encircled the epitope to the 29 amino acids N-terminal by the recombinant fragments. The epitopes of IG 12 and Bo 14 could be limited to amino acid residues 48-53 and 226-232, respectively. Further definition of the epitopes was performed by synthesizing peptides spanning the respective epitopes and mutating each amino acid position by the 20 possible amino acid residues. Thus, the essential amino acids for the antibody reactivity were elucidated. From these data we further can deduce to which grass species and group 1 isoforms the antibodies will bind.

Our results demonstrate clearly definable linear epitopes for the monoclonal antibodies IG 12 and Bo 14, while the HB 7 epitope seems to span a wider region probably due to conformational segments. These studies will help to build up assays for monitoring and to isolate group 1 allergens by affinity chromatography.

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A strategy to discriminate between IgE responses to cross-reactive carbohydrate determinants and allergen-specific epitopes

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Background: The widespread presence of cross-reactive carbohydrate determinants (CCD) on allergens from plants and invertebrates and their capability to induce cross-reactive IgE has been given considerable attention. All *in-vitro* methods measuring specific IgE based on native allergens will also, if present, measure CCD-specific IgE with non- or weak clinical significance. Bromelain from pineapple stem has been suggested to carry one of the most general CCD structures and is also known to rarely induce clinically relevant allergy reactions. Bromelain could therefore be useful for studying CCD-specific IgE.

Objective: To define strategies to discriminate CCD-specific from peptide IgE responses.

Methods: Thirteen potentially CCD-reactive human sera with concurrent IgE reactivity to a panel of allergens known to carry CCD-structures and bromelain, were selected. Inhibition of the IgE reactivity using a CCD-inhibitory reagent based on bromelain was performed in the ImmunoCAP™ system and with Immunoblotting.

Results & Discussion: The CCD-inhibitory reagent inhibited the reactivity of all sera to the allergens in the panel, demonstrating the presence of CCD-IgE. However, the level of inhibition differed between the allergens. Inhibition studies of timothy and mugwort specific IgE revealed two different types of inhibition patterns. The first category involved sera that had a higher IgE reactivity to the allergen than to bromelain and included 6 sera which were inhibited 20- 40% on the timothy ImmunoCAP™. This reactivity most likely consisted of a mixed population of both CCD-IgE and timothy-specific IgE. The second category comprising sera that had a similar response level to the allergen and bromelain included 7 sera which were inhibited 40-100% on the timothy ImmunoCAP™. When analysed on the mugwort ImmunoCAP™, all 13 sera, were classified as the second category and the observed reactivity most likely predominantly consisted of CCD-IgE.

Conclusion: By combining ImmunoCAP™ results for allergens carrying CCD-structures and the results from the inhibition studies it was possible to estimate the contribution of CCD-IgE to the total reactivity to timothy and mugwort in 13 human sera. This strategy could be of great importance when evaluating the clinical relevance of positive results for CCD-containing allergens as well as the clinical significance of IgE specific for CCD-structures.

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Relevance of carbohydrate determinants for the differentiation between true latex allergy and asymptomatic IgE reactivity

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IgE-binding to cross-reacting carbohydrate determinants (CCDs) has already been described for several plant proteins, but their relevance for latex allergy is yet to be determined. Natural rubber latex (NRL) allergens, such as Hev b 2 and Hev b 13, causing latex allergy in health care workers (HCW) and spina bifida (SB) patients are glycosylated proteins. Although the risk to develop a latex allergy is declining in high-risk groups, sensitization outside of the medical field was observed. Discrimination between true latex allergy and clinically insignificant IgE sensitization still poses a diagnostic challenge. The IgE reactivity to single NRL allergens and horseradish peroxidase (HRP) used as a marker of CCDs was analyzed with the UniCAP-system using sera from subjects demonstrating NRL-specific IgE. These included 47 latex-allergic HCWs, 20 latex-allergic SB patients, 11 patients with allergy to hymenoptera venoms and 32 workers of a seafood processing company. Three of the 47 HCW sera with clinically significant NRL allergy had elevated specific IgE to HRP. Although more than 80% of the HCW sera recognized native Hev b 2, the binding of NRL-specific IgE antibodies was not inhibited by HRP. None of the SB patients' sera demonstrated IgE-binding to HRP. 30 out of 32 seafood workers' sera randomly selected from 63 latex-sensitized workers displayed comparable specific IgE responses to HRP and NRL (HRP-specific mean: 3.12 kU/L; NRL-specific mean: 2.7 kU/L). The reactivity to the recombinant allergens was low and in only three cases Hev b 8-specific IgE was detected. The prevalence of Hev b 2-specific IgE was about 90% and mostly the Hev b 2-specific IgE-values were similar to the HRP-values. Inhibition of NRL-specific IgE-binding with HRP was >80%. Furthermore, 9 of 11 sera of patients with hymenoptera venoms allergy and latex sensitization showed HRP-specific IgE, but no specific IgE to Hev b 5 or Hev b 6.01. In these cases the NRL-specific IgE-binding was completely abolished in the presence of HRP. In conclusion, our data indicate that reactivity to CCDs such as HRP could be used as an *in vitro* screening tool for differentiating true latex allergy from clinically insignificant elevated IgE to NRL.

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The molecular basis of IgE-mediated autoreactivity

Crameri Reto

Direct screening of a human lung cDNA library displayed on phage surface with solid-phase immobilized serum of patients suffering from long lasting allergic asthma and atopic eczema revealed hundreds of complete and partial sequences potentially encoding IgE-binding self-antigens. Among these, MnSOD, ribosomal P_2 protein, thioredoxin, heat shock proteins, and cyclophilin, show relevant sequence identity to common phylogenetically conserved environmental allergens. The recombinantly produced human antigens show the expected enzymatic activity, and thus native like folding, and have been demonstrated to induce specific T cell proliferation in PBMC's of sensitized individuals, to bind IgE in ELISA and Western blot analyses *in vitro*, and to induce immediate Type I hypersensitivity reactions in skin tests. These reactions, so far only observed in chronic inflammatory forms of allergy like asthma, atopic eczema and occupational exposure, are likely to contribute to the perpetuation of the symptoms. We have now solved the crystal structure of MnSOD (Asp f 6) and cyclophilin (Asp f 11) of *Aspergillus fumigatus,* as well as the crystal structures of cyclophilin (Mala s 6) and thioredoxin (Mala s 13) of *Malassezia sympodialis*. Extensive comparisons of these crystal structures with the solved crystal structures of the corresponding human enzymes revealed conserved folds and patches of conserved amino acids scattered over the whole linear sequences of the molecules which become assembled on the surface forming potential conformational IgE-binding epitopes. In contrast to the extended sequence identity at primary structure level, only 10-15% of the identical amino acids of the molecules are solvent exposed and thus able to contribute to antigen/antibody interactions.

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Acid-suppresssing drugs applied to pregnant females sensitize the mother and support a perinatal Th2-environment in the offspring of BALB/c mice

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Background: About 70% of pregnant women suffer from reflux and heartburn. The first-line treatments are antacids or sucralfate. However, anti-acid therapeutics have been shown to interfere with

the peptic digestion of food proteins and thereby promote oral sensitization. Therefore, these substances may affect the immune response to food proteins in the mother and the offspring.

Objective: To clarify the effect of sucralfate-treatment of the mother during pregnancy and lactation on the food-specific immune response in the offspring in a BALB/c mouse model.

Methods: Dams were fed codfish extract plus sucralfate during their late pregnancy and lactation. To investigate the influence of sucralfate-treatment on the sensitization against homologous and heterologous antigens, the offspring was simultaneously injected codfish and ovalbumin intraperitoneally. Antigen-specific antibodies were determined in serum of mother mice and young animals. Cytokine levels in supernatants of antigen-stimulated splenocytes and skin reactivity of the offspring were evaluated.

Results: The mother animals revealed high codfish-specific IgG1 and positive skin tests, which indicated an allergic response against codfish induced via sucralfate-treatment. Also in their offspring high amounts of codfish-specific IgG1 were found during the suckling period and already before sensitization, likely being of maternal origin. After these young animals were sensitzed i.p. with codfish and ovalbumin at different time intervals after birth, no differences were seen regarding the amounts of antigen-specific IgG1 or IgG2a. However, the induction of codfish-specific (but not ovalbumin-specific) IgE was significantly inhibited. Despite this suppression, the amount of IL-5 compared to IFN-³ was much higher in the offspring of mothers treated with sucralfate. This Th2-biased immune response was paralleld by a significant reduction of IL-10 and enhanced skin test reactivity.

Conclusion: From our data we conclude that sucralfate-treatment induces a Th2-response in the mother. When these maternal antibodies are transferred to the offspring, they suppress antigen-specific IgE synthesis. On the other hand, we observed that anti-acid drugs support the development of a Th2 environment in newborns.

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Vacuolar serine proteases from *Cladosporium herbarum* and *Alternaria alternata*

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Background: *Cladosporium herbarum* and *Alternaria alternata* represent one of the most prominent fungal species causing Type I Allergy. Previously, several allergens have been cloned from these molds. Nearly all of these allergens, except Alt a 1, the major *A. alternata* allergen, have been isolated from both molds and were shown to be IgE-cross-reactive. Since vacuolar serine proteases have been described as cross-reactive allergens from various fungal species (*A. fumigatus*, *A. niger*, *P. chrysogenum*, *P. oxalicum*, and *R. mucilaginoasa*), it was very likely that there exist homologous allergens in *C. herbarum* and *A. alternata*.

Methods: cDNA-expression libraries from *C. herbarum* and *A. alternata* were screened with a cDNA clone coding for the vacuolar serine protease from *P. oxalicum*.

Results: Screening of the *C. herbarum* cDNA library resulted in a full-length clone of 1661bp coding for a 519 amino acids long pre-pro-protein. The isolated clone shows a sequence identity of 67.9% with Pen o 18. Testing of the IgE-reactivity revealed that four out of 20 patients (20%) reacted with the *C. herbarum* vacuolar serine protease (Cla h 9). In case of *A. alternata* a partial clone of 790bp has been isolated so far. Previously, two monoclonal antibodies (FUM20 and PCM39), which were generated against culture medium and/or crude extract from *P. citrinum* and *A. fumigatus*, were shown to cross-react with the vacuolar serine proteases from *P. notatum*, *P. oxalicum* and *A. fumigatus*. Performing IgG-immunoblots we could show that FUM20 and PCM39 also cross-react with Cla h 9.

Conclusion: The vacuolar serine proteases from *C. herbarum* and *A. alternata* represent two new allergens. Cla h 9 has been shown to be cross-reactive on the IgE-level with *P. oxalicum* and on the IgG- level with *P. notatum*, *P. oxalicum* and *A. fumigatus*, respectively.

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Cloning of new *Dermatophagoides pteronyssinus* allergens for diagnosis and therapy of house dust mite allergy

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House dust mites, in particular *Dermatophagoides pteronyssinus,* belong to the most important allergen sources worldwide against which 10% of the population and more than 50% of allergic patients are sensitized. We have constructed a Der p expression cDNA library in lambda gt11 and screened it with IgE antibodies from mite allergic subjects. cDNAs coding for two new mite allergens, designated clone 25 and clone 30-derived allergens, were isolated, expressed in *Escherichia coli* and purified to homogeneity.

The clone-25 derived allergen represented a 14 kDa protein with predominantly α-helical secondary structure, high thermal stability and refolding capacity. The mature protein bound IgE from 26% of mite allergic patients and in certain histamine release experiments was biologically more active than the major house dust mite (HDM) allergen, Der p 1. The clone 25-derived allergen revealed significant homology with group 5 allergens of different mite species, but IgE cross-inhibition studies and binding studies performed with an antiserum raised against the recombinant clone 25-derived protein indicated lack of cross-reactivity with Der p 5 and with allergens from storage mites. By immunogold electron microscopy, the clone 25-derived allergen could be located in the midgut of *D. pteronyssinus* where it was associated with the gut endothelium, the lumen and feces.

The cDNA of clone 30 coded for another new allergen with a molecular weight of 8 kDa which showed significant homology with the chitin-binding domain type 2. It bound IgE antibodies from more than 50% of mite allergic patients, hence representing a major HDM allergen.

The newly identified, recombinant Der p allergens complement the existing panel of HDM allergens and may be useful for diagnosis and immunotherapy of house dust mite allergy.

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Prostate-Specific Antigen as Allergen in Human Seminal Plasma Allergy

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Seminal plasma allergy is increasingly being recognized. Its implications and impact on sexuality and reproduction are significant, and it may also cause life-threatening anaphylactic reactions.

Here we present the case of a 31-year-old woman, who complained about recurrent rhinitis, asthma attacks and conjunctivitis 30-45 minutes after sexual intercourse. The patient had a 10 years history of asthma with multiple sensitizations against aeroallergens and latex. Sexual intercourse-related symptoms could be prevented by latex-free condoms.

Allergy diagnosis showed specific IgE to seminal plasma (CAP-class 2) and positive skin prick test reactions (SPT) to 1:2, 1:4, 1:16 and 1:160 dilutions of her husband's seminal plasma, but not to the spermatozoa.

As first therapeutic approach an intravaginal rush desensitization with diluted husband's seminal plasma was attempted. Thereafter, the patient had repeated unprotected sexual intercourse at intervals of 2-3 days with slight local itching and vulvar swelling only. However, two weeks after the desensitization attempt, the patient experienced generalized urticaria with respiratory distress 30 minutes after sexual intercourse with subsequent emergency treatment. Specific IgE against seminal plasma had increased to 4.27 kU/l (CAP-class 4), and positive SPT reactions against seminal plasma could be observed up to a dilution of 1:10000.

In order to examine, which components of the husband's seminal plasma are recognized by IgE antibodies of the patient, we performed SDS-PAGE followed by Western Blotting. A clear band of about 33 kDa was visible suggesting that prostate-specific antigen (PSA) might be the predominant allergen. Indeed commercial, highly pure human PSA also yielded a strong band when probed with the patient's serum. After preabsorption of the patient's serum with pure PSA the immunoreactive bands were absent. High leukotriene release (CAST®) and more than 50% basophil activation (Basotest®) could be observed after incubation with of the husband's seminal plasma (1:10- 1:160) as well as PSA (0.04/0.02/0.01 µg/ml).

Together these results strongly suggest that PSA was selectively recognized by IgE antibodies contained in the patient's serum.

It might be speculated whether in the presented patient successful desensitization could be achieved using purified PSA obtained from the husband's seminal plasma or serum.

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Mechanisms of immune tolerance to high dose allergen exposure in healthy individuals

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Natural ways for the induction and life span of T regulatory cells have been an essential question in this rapidly developing area. We have demonstrated in healthy individuals that allergen-specific Th1-like cells, Th2-like cells and IL-10-secreting Tr1 cells exist in different quantities and their ratio is decisive for the development of allergic or healthy immune response. Here, we investigated allergen-specific T cell tolerance in healthy beekeepers, who receive multiple bee stings due to their occupation. Compared to aeroallergen exposure, this model is better controlled and represents a much higher dose of exposure via skin. These individuals have been followed for several years and as soon as they received multiple bee stings at the beginning of each bee-keeping season, they showed very significant T cell tolerance within 3 to 7 days as determined by abolished proliferative response to the bee venom major allergen phospholipase A_2. Peripheral T cell tolerance lasts a few months after the bee stings and returns to initial levels out of the season every year. In addition, changes in the frequency of T cell subsets have been analysed by using allergen-specific T cell purification and ELISPOT analysis, which showed that allergen-specific Tr1 cells significantly increased, Th1 and Th2 cells decreased within 7 days. In parallel, allergen-induced IL-10 secretion increased and IL-4, IL-13 and IFN-γ secretion decreased. The analysis of suppressor factors revealed that in addition to previously demonstrated IL-10, CTLA-4 and PD-1; histamine receptor 2 (HR2) was immediately upregulated in Tr1 cells and played an essential role in allergen tolerance. Triggering of HR2 in Th2 and Tr1 cells significantly upregulated IL-10 production, demonstrating a feed-back loop for the enhancement of T regulatory cell activity. In contrast, blocking of HR2 partially abrogated the suppressor function of Tr1 cells. These data were supported in a study of phospholipase A_2 peptide immunotherapy, which showed significantly increased histamine receptor 2 expression in allergen-specific T cells. In conclusion, the rapid generation capacity of allergen-specific Tr1 cells rather than their long life span and their use of multiple suppressor factors represent decisive mechanisms of immune tolerance to allergens in healthy individuals.

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Evaluation of Safety and Clinical Efficacy of a Higher Dose Regimen of a Ragweed Amb a 1 Immunostimulatory Conjugate for Treatment of Patients with Ragweed-induced Seasonal Allergic Rhinitis

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We have shown that chemical modification of the principal protein moiety of ragweed (Amb a 1) with immunostimulatory DNA (CpG motif) provides a therapeutic construct that can be given safely with a brief 6-injection (inj) regimen and offers measurable clinical efficacy that persists through two seasons.

We now report the findings of a randomized, double-blind, placebo (PL) -controlled, pilot study of a 7-inj [n=9 (AIC:6, PL:3): 0.3/1.2/3.0/ 6.0/15/30/ 30µg] or a 6-inj [n=9 (AIC:6, PL:3): 1.2-30µg] regimen in RW allergic rhinitics that assessed safety, IgG/E antibody response, skin test reactivity and clinical outcomes. All study patients (pts) completed their injections and reached the final dose [Cohort I: 65 inj (cumulative dose: 85.2µg); Cohort II: 54 injections (cumulative dose: 85.5µg)]. AIC was well tolerated and no serious adverse events were observed.

AIC induced a significant rise in IgG anti-Amb a 1 antibody in 11/12 pts

(e" 2-fold increase in 7/12 pts). AIC did not induce a rise in IgE anti-Amb a 1 and treatment blunted the expected seasonal rise in RW IgE. AIC suppressed the late phase intradermal skin test reaction post RW season [AIC: -27.5 mm, PL: +5 mm (p=0.008)]. Visual analog scores recorded during the peak RW season demonstrated that PL-treated pts experienced a significant increase in symptoms [+ 11.5 vs baseline (p=0.04)]; whereas AIC-treated pts did not show a rise in symptoms [-1.75 vs baseline]. The difference between the two treatment arms was 13.25 units (p=0.052). The AIC pts also used less relief medications during the peak RW season.

Based on these observations, a two year multi-center clinical trial was initiated in 462 RW allergic pts [AIC (n=310), PL (n=152)]. In this clinical protocol a weekly 6-inj regimen [1.2/3.0/6.0/15/21/30µg] with a booster phase prior to the second RW season [3.0/30µg (1 week apart)] was administered. The results of this clinical study will also be reviewed.

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Peptide therapy reduces allergic lung inflammation through the induction of IL-10 secreting CD4+ T cells and through deletion of peptide-specific effector T cells in an HLA-DR1 transgenic mouse model

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Rationale: Cat allergic individuals develop clinical tolerance to both cat peptide and whole cat dander challenge following intradermal (i.d.) treatment with peptides representing T cell epitopes from the major cat allergen Feld1. To investigate mechanisms of peptide-induced tolerance, we have developed a model of cat allergen sensitivity in HLA DRB1*0101-transgenic, endogenous MHC class II knockout mice.

Methods: Mice were sensitised by intraperitoneal injection of Feld1 in alum followed by intranasal cat allergen challenge and a single therapeutic i.d. treatment with active or control peptide, prior to intra-nasal allergen re-challenge. After lung function analysis, mice were sacrificed and lung inflammation assessed. The fate of T cells specific for the treatment peptide was followed using a novel HLA-DR1 tetramer.

Results: Mice treated with Feld1 peptide demonstrated a significant reduction in airway hyperreactivity (p=0.05). Peptide-treated mice had lower eosinophil numbers in BAL (p=0.01) and lung digest (p=0.01). Expression of Il-4, IL-5 and IL-13 were significantly reduced in Feld1 peptide-treated mice compared to controls. Levels of IL-10 in BAL were increased (p<0.001) and IL-10+ CD4 T cells in lung digests increased almost 3-fold. CD4 T cells specific for the treatment peptide were markedly decreased, but not eliminated, after peptide therapy. Tetramer+ cells were not detected in draining lymph nodes.

Conclusions: These findings indicate that i.d. Feld1 peptide treatment reduces allergic lung inflammation in pre-sensitised HLA-DR1 transgenic mice. Additionally, the results suggest reduced inflammation may be associated with the induction of IL-10 producing CD4+ T cells and deletion or sequestration of T cells specific for the treatment peptide. Human HLA-DR1 transgenic mice thus provide an informative model to investigate mechanisms of peptide-induced tolerance to cat allergen.

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Longitudinal Study of Sublingual Immunotherapy with SQ Standardized Grass Allergen Tablets – Interim Efficacy Results

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Rationale: Specific immunotherapy with sublingual drops (SLIT) is today a widely used treatment for respiratory allergies with the aim to relieve symptoms by treating the cause of the allergic disease. SLIT is a safe and efficacious treatment, and the number of controlled clinical studies documenting the clinical benefits of SLIT is constantly growing.

The clinical efficacy of a fast dissolving grass allergen tablet (GRAZAX®, ALK-Abelló A/S) for sublingual use has been evaluated in the largest clinical development program in sublingual immunotherapy ever performed. Previous safety and dose finding trials have proposed a daily dose of 75000 SQ-T taken at home as a once daily tablet with no up-dosing to be an optimal dose in terms of clinical efficacy, safety and tolerability. In addition the importance of a sufficient pre-seasonal treatment period has been established as a critical factor to obtain clinical efficacy already in the first treatment season.

Confirmatory efficacy data from the first pre- and co-seasonal period of an ongoing long term trial are presented.

Methods: In a longitudinal, double-blind, placebo-controlled, parallel-group trial including 51 centers from 8 European countries patients were randomized (1:1) to receive either Grazax 75000 SQ-T or placebo once daily. Following a pre- and co-seasonal treatment patients were asked to continue double-blind treatment for additionally 2 years, followed by a 2-year follow-up period. 634 subjects with a history of mild to moderate grass pollen induced rhinoconjunctivitis for at least 2 years and laboratory confirmation (SPT and serum-specific IgE), were included in the trial. Treatment was initiated 4-6 months prior to the grass pollen season and continued throughout the season. Primary endpoints were rhinoconjunctivitis symptom and medication scores.

Results: A 30% reduction in rhinoconjunctivitis symptoms and a 38% reduction in symptom relieving medication were found in addition to the placebo effect. Both reductions were highly statistically significant ($p<0.0001$). The treatment was well tolerated raising no safety concerns

Conclusions: These data confirms the clinical efficacy and safety of Grazax in an optimal dose of 75000 SQ-T sublingual tablets taken once daily as self-medication with no up-dosing. The clinical efficacy was significant already in the first treatment season. The double-blind placebo-controlled extension of this trial will provide data concerning the efficacy during the following seasons.

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Induction of "regulatory" T cells by Cynodon dactylon (Bermuda grass) specific immunotherapy

Robyn O'Hehir

Rationale: We have previously implicated excessive activity of the transcriptional regulatory factor AP-1, a heterodimer of c-fos and c-jun which promotes the transcription of several asthma-relevant cytokine genes, in the regulation of the clinical response of asthmatics to systemic glucocorticoid (GC) therapy. Activity of c-jun is regulated through phosphorylation by c-jun kinase (JNK), which is itself activated through phosphorylation by JNK kinase. We hypothesised that the expression and phosphorylation status of c-jun and JNK are increased in cells of the bronchial mucosa of GC resistant, as compared with sensitive asthmatics and are differentially regulated by systemic GC therapy.

Methods: We used immunohistochemistry with validated antibodies and digital image analysis to measure the expression of c-fos, total and phosphorylated c-jun (c-jun P) and total and phosphorylated JNK (JNK P) in mucosal infiltrating cells in bronchial biopsies from 9 GC sensitive (mean δ FEV_1 40.0 ± 39.7%, age 35-67 yr) and 17 GC resistant (mean δ FEV_1 0.3 ± 3%, aged 25-73 yr) asthmatics taken before and after 2 wks of therapy with oral prednisolone (1.72mg/m^2 body surface area). Total leukocytes were numerated using the pan-leukocyte marker CD45.

Results: Median total numbers of (CD45) mucosal leukocytes were not altered by GC therapy in either group. At baseline, median total numbers of c-jun immunoreactive cells were elevated in the GC resistant, as compared with the sensitive asthmatics ($p=0.015$). In addition, median total numbers of c-jun P immunoreactive cells ($p=0.004$), the fraction of c-jun+ cells in which c-jun was phosphorylated ($p=0.008$) and the numbers of cells showing immunoreactivity for JNK P were significantly reduced, starting from a similar baseline, following prednisolone in the GC sensitive, but not the resistant asthmatics ($p=0.039$). Median numbers of c-fos immunoreactive cells were similar in both groups of baseline, and did not significantly change following prednisolone.

Conclusions: Clinical GC responsiveness in asthma is accompanied by reduced phosphorylation of bronchial mucosal c-jun, a phenomenon not seen in resistant patients. Persistent activation of the c-jun kinase chain, possibly partly by external environmental factors, may play a role in the clinical refractoriness of asthmatics to GC therapy *in vivo*.

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Systemic glucocorticoid fails to inhibit the c-jun/c-jun kinase phosphorylation cascade in bronchial mucosal cells of glucocorticoid resistant asthmatics

Tak Lee, Brian O'Connor, Jonathan Ratoff, Kirsty Mallett, Tuck-Kay Loke, Chris Corrigan

***Rationale*:** We have previously implicated excessive activity of the transcriptional regulatory factor AP-1, a heterodimer of c-fos and c-jun which promotes the transcription of several asthma-relevant cytokine genes, in the regulation of the clinical response of asthmatics to systemic glucocorticoid (GC) therapy. Activity of c-jun is regulated through phosphorylation by c-jun kinase (JNK), which is itself activated through phosphorylation by JNK kinase. We hypothesised that the expression and phosphorylation status of c-jun and JNK are increased in cells of the bronchial mucosa of GC resistant, as compared with sensitive asthmatics and are differentially regulated by systemic GC therapy.

***Methods*:** We used immunohistochemistry with validated antibodies and digital image analysis to measure the expression of c-fos, total and phosphorylated c-jun (c-jun P) and total and phosphorylated JNK (JNK P) in mucosal infiltrating cells in bronchial biopsies from 9 GC sensitive (mean ??FEV_1 40.0 ± 39.7%, age 35-67 yr) and 17 GC resistant (mean ??FEV_1 0.3 ± 3%, aged 25-73 yr) asthmatics taken before and after 2 wks of therapy with oral prednisolone (1.72mg/m^2 body surface area). Total leukocytes were numerated using the pan-leukocyte marker CD45.

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***Conclusions*:** Clinical GC responsiveness in asthma is accompanied by reduced phosphorylation of bronchial mucosal c-jun, a phenomenon not seen in resistant patients. Persistent activation of the c-jun kinase chain, possibly partly by external environmental factors, may play a role in the clinical refractoriness of asthmatics to GC therapy *in vivo*.

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Association between persistent wheeze and specific IgE, IgG and IgG4 antibodies

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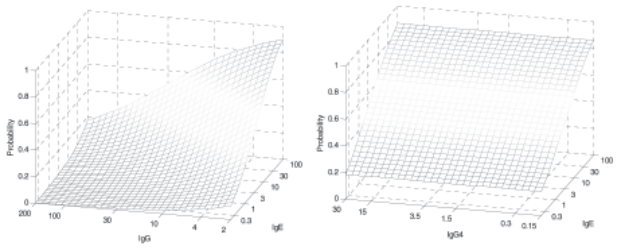
Background. We have previously demonstrated that the probability of wheeze in childhood increases with increasing specific IgE antibody levels (Simpson et al, JACI 2005). It is unclear whether allergen-specific IgG antibody responses modify the association between IgE and allergic respiratory symptoms.

Aim: Within the context of a population-based birth cohort study, we investigated the association between specific IgE, IgG and IgG_4 antibodies with persistence of wheeze in 5 year-old children.

Methods: Children (n=483) were followed from birth to age 5 years. According to parentally-reported history of wheeze at the follow-ups at age 3 and 5 years, children were assigned as persistent wheezers if they had at least one episode of wheezing during the first three years of life and reported wheezing in the previous 12 months at age 5 years (n=70). Specific serum IgE to cat was measured using UniCAP™ assay. In addition, we measured Fel d 1-specific IgG and IgG_4 antibodies (UniCAP™).

Results: The predicted probability of persistent wheeze increased 1.75-fold (95% CI 1.35-2.26, p<0.001) per logarithmic unit increase in cat IgE antibody level. However, this effect was significantly modified by Fel d 1-specific IgG antibodies (the magnitude of the effect of specific IgE decreased with increasing Fel d 1-specific IgG; Figure A). Allergen-specific IgG_4 had no effect on symptoms (Figure B). In a multivariate logistic regression analysis which included IgE, IgG and IgG_4, cat-specific IgE and Fel d 1-specific IgG remained significant and independent predictors of persistent wheeze. In this model, predicted probability of persistent wheeze increased 1.88 fold (95% CI 1.38-2.56, p<0.001) per logarithmic unit increase in cat IgE antibody level, but decreased significantly with increasing Fel d 1-specific IgG (OR 0.41, 95% CI 0.28-0.61, p<0.001). IgG4 was not associated with persistent wheeze (1.19, 0.89-1.59).



Conclusions: The probability of persistent wheeze increases with increasing cat specific IgE. This association is significantly modified by Fel d 1-specific IgG antibodies, with the risk of symptoms decreasing with increasing Fel d 1-specific IgG. Fel d 1-specific IgG_4 had no effect on symptoms.

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Mechanistic Studies of Tumour Necrosis Factor Alpha in Asthma

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Rationale: We have shown that treatment of patients with chronic severe asthma with the soluble TNF± receptor-IgG1Fc fusion protein, etanercept (Enbrel®) improves asthma control, lung function and BHR (*Howarth et al. Thorax 2005 Sep 15; Epub ahead of print*). Here we describe mechanistic studies of the role(s) of TNF-± in asthma.

Methods: 9 patients with mild house dust sensitive asthma underwent repeated low dose allergen exposure and had bronchial biopsies before and after allergen exposure. These were assessed by immunohistochemistry (IHC) for presence of inflammatory cells, TNF-±, ICAM-1 and VCAM-1. A further 12 subjects with moderately severe asthma underwent bronchoscopy and the biopsies were cultured for 24h as tissue explants to assess the expression of TNF-±, ICAM-1 and IL-8 in the presence of *Der p* and CDP870, a neutralizing anti-human TNF-± Fab antibody fragment. Supernatants were analysed by ELISA and tissue fixed for IHC.

Results: Following low dose allergen inhalation, sub-mucosal mast cells increased from 19.8cells/mm^2 (range 13.0-30.3) to 33.3cells/mm^2 (21.8-51.1), p=0.018. There was also upregulation of TNF-± from 0.46cells/mm^2 (0.19-1.40) to 1.63cells/mm^2 (0.76-4.91), p=0.018 following allergen exposure. Submucosal VCAM and ICAM expression increased from 0% to 9.72% (5.33-71.28), p=0.046 and from 48.49% (32.77-59.02) to 85.63% (67.31-100.0) respectively.

As in the *in vivo* study, *in vitro* exposure of bronchial explants to *Der p* caused significant upregulation of TNF-± from 1.1 (0.2-1.6) to 2.4cells/mm^2 (0.9-7.1), p=0.02; this decreased to 0.2cells/mm^2 (0.0-0.5) in the presence of the blocking antibody. *Der p* also increased submucosal expression of ICAM-1 from 79.0 (62.05-86.72) to 85.65% (75.49-99.63) and this was decreased in the presence of CDP870 (59.89% (36.52-80.12), p=0.01). In the explant supernatants, levels of TNF-± were unchanged in the absence (11.3 (5.9-19.5) pg/mg) or presence of *Der p* (8.8 (5.1-30.2) pg/mg; p=NS). However, CDP 870 decreased TNF-± levels to 1.6 (0.2-6.9) pg/mg, p<0.01 and IL-8 levels from 28.1(13.0-39.0) in the presence of *Der p* to 26.4 (14.0-34.7) pg/ml, p=0.05.

Conclusions: These studies identify several potential roles for TNF-± as a mediator in asthma. Blocking TNF-± may suppress inflammation by decreasing IL-8 and adhesion molecule expression. It may also prevent autocrine stimulation of mast cells.

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Mechanotransduction of the UPA-UPAR in airway epithelial cells

Jeffrey Drazen

Mechanical stimulation of the airway epithelium, as would occur during bronchoconstriction is a potent stimulus and can activate profibrotic pathways. We used DNA microarray technology to examine gene expression in compressed normal human bronchial epithelial cells (NHBE). Compressive stress applied continuously over an 8 hour period to NHBE cells led to the upregulation of several families of genes including a family of plasminogen related genes that were previously not known to be regulated in this system. Real-time PCR demonstrated peak gene expression of 8.0 fold for urokinase plasminogen activator (uPA), 16.2 fold for urokinase plasminogen activator receptor (uPAR), 4.2 fold for plasminogen activator inhibitor-1 (PAI-1) and 3.9 fold for tissue plasminogen activator (tPA). Compressive stress also increased uPA protein levels in the cell lysates (112.0 vs 82.0 ng/ml, p=0.0004), and increased uPA (4.7 vs 3.3 ng/mL p=0.02), uPAR (1.3 vs 0.86 ng/mL p=0.007) and PAI-1 (50 vs 36 ng/mL p=0.006) protein levels in cell culture media. Functional studies demonstrated increased urokinase dependent plasmin generation in compression stimulated cells (0.0090 vs 0.0033 OD/min, p=0.03). In addition, compression led to increased activation of matrix metalloproteinase (MMP)-9 in a urokinase dependent manner. Thus mechanical stimulation of NHBE cells through its effects on the plasminogen activating system is capable of modifying the airway micro-environment.

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Helminth infection induces regulatory T cells and inhibits allergen-mediated sensitization and airway disease in a murine asthma model

Eckard Hamelmann

Background: Numerous epidemiological studies have shown an inverse correlation between helminth infections and the manifestation of atopic diseases, yet the immunological mechanisms governing this phenomenon are indistinct. **Objective:** We therefore investigated the effects of infection with the filarial parasite *Litomosoides sigmodontis* on allergen-induced immune reactions and airway disease in a murine model of asthma. **Methods:** Premature female *Litomosoides sigmodontis* filaria were implanted intraperitoneally prior to allergic sensitization and challenge with ovalbumin. Systemic sensitization, airway and airway hyperreactivitywere determined to assess effects on the asthmatic phenotype. Flow cytometry and cytokine-specific ELISAs were used to analyze changes in immune phenotype. **Results:** Infection with *L. sigmodontis* suppressed all aspects of the asthmatic phenotype: antigen-specific immunoglobulin production, airway reactivity to inhaled methacholine, and pulmonary eosinophilia. Similarly, antigen-specific recall proliferation and Th2 cytokine production were significantly reduced after *L. sigmodontis* infection. Flow cytometry analysis of spleen cells revealed a significant increase in numbers of T cells with a regulatory phenotype ($CD4^+/CD45RB^{low}/CD25^+$) in infected and sensitized mice compared to sensitized controls. Additionally, surface and intracellular staining for TGF-² as well as antigen-specific TGF-² production was increased in infected and sensitized animals and locally in mediastinal lymph nodes *Foxp3* expression levels were substantially increased, denoting an increase in regulatory T cells in this compartment as well.

Conclusion: These data strongly support the epidemiological evidence concerning the impact of helminth infections on atopic diseases pointing towards the induction of regulatory T cells and enhanced secretion of the immunomodulatory cytokine TGF- ² as an underlying mechanism.

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CD48 is critically involved in experimental asthma

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Rationale: CD48 is a glycosylphosphatidylinositol-anchored protein belonging to the CD2-subfamily. It is expressed mainly on hematopoietic cells and exists both as a membrane-associated and a soluble form. Studies on CD48 deficient mice indicate that CD48 has a broad range of immunological functions. it interacts with extracellular matrix components such as heparan sulfate, functions as an adhesion molecule and provides co-stimulatory signals to T and B lymphocytes. Furthermore, CD48 has a distinctive role in mast cell innate immune responses to E. coli.

Objective: To investigate the role of CD48 in asthma using an experimental mouse model

Methods: Asthma was induced in mice by OVA/Alum sensitization and intranasal inoculation of OVA or induced by repeated intranasal *A. fumigatus* antigen in wild type Balb/c; STAT-6 deficient; IL-4/IL-13 deficient; and IL-5/eotaxin-1 deficient mice. Gene profiling of whole lung was performed (Affymetrix U74Av2 Gene Chip) followed by Northern blot analysis. Balb/c mice were treated with a long acting form of IL-3. Differential cell counts and analysis of CD48 expression (FACS) were performed on splenocytes with cells from bronchoalveolar lavage (BALF). Anti-CD48 was administered before OVA challenge and BALF was assessed for differential cell counts and cytokines. Lungs were fixed, sectioned and stained with H&E, PAS and Masson's trichrome.

Results: Microarray analysis demonstrated increased expression of CD48 in the lungs of allergen challenged mice. Northern blot analysis of total lung from various knockout mice indicated that CD48 upregulation was STAT-6, IL-13, IL-4, IL-5 and eotaxin-1 independent. IL-3 administration caused a significant eosinophilic- and basophilic-infiltration into the spleen, BALF and lungs and upregulated CD48 expression on eosinophils and basophils. Additionally, inducible IL-13Tg mice expressed elevated levels of CD48. Neutralization of CD48 in allergen-challenged mice resulted in abrogation of lung inflammation and remodeling, diminished cytokine/chemokine levels, and decreased eosinophilia in the BALF. Moreover, CD48 was found to be upregulated on eosinophils from atopic asthmatics in comparison to normal healthy controls.

Conclusions: Our results define a novel pathway that is critically involved in the orchestration and regulation of experimental asthma. Therefore, we suggest CD48 as a new target for future therapeutic approaches to treatment of allergic airway inflammatory disease.

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The Role of NKT Cells in the Development of Asthma

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We examined the role of NKT cells in the development of allergen-induced airway hyperreactivity (AHR). We found that in the absence of NKT cells, in CD1d$^{-/-}$ mice (which lack the restricting element of NKT cells) or in Jα281$^{-/-}$ mice (which lack the invariant TCR of NKT cells), allergen-induced AHR failed to develop. The failure to develop AHR was due specifically to the absence of NKT cells since reconstitution of the Jα281$^{-/-}$ mice by adoptive transfer of NKT cells isolated from wild type BALB/c mice reconstituted the development of AHR.

The precise mechanisms by which NKT cells induce AHR are not clear. Th2 responses can occur in NKT cell deficient mice, suggesting that Th2 responses are necessary but not sufficient for the development of AHR. This is consistent with the observation in humans that not all individuals with allergic rhinitis develop asthma, suggesting that NKT cells must regulate additional elements in the lower respiratory tract that are required for the development of AHR. In patients with moderate to severe asthma, we found large number of NKT cells present in bronchial lavage fluid, suggesting that NKT cells play a very important role in human asthma. We suggest that NKT cells in the lung become activated by glycolipid antigens encountered in the lung and provide a necessary prerequisite for the development of AHR, perhaps by localizing the Th2 response to the lungs or interacting synergistically with Th2 cells that enter the pulmonary compartment. Thus IL-4 and IL-13 produced by NKT cells potentiate the development and action of Th2-biased T cells in the lungs, and together these cells drive the development of asthma.

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IgE expression is regulated by alternative polyadenylation

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In vitro data of our group and other showed a fundamental difference between IgE and other immunoglobulins in the expression pattern of mRNA for the secreted and membrane form. Responsible for this difference is the poor expression or poor stability of the mRNA for the membrane form of IgE. We decided to construct two reporter mouse strains, carrying chimaeric IgE/IgG1 antigen receptors. In the first "knock-in I" we completely exchanged the 3´sequences downstream of spoly(A) of IgE to IgG1 type. In the second "knock-in II" only the exons (M1 and M2) were exchanged for IgG1 type by leaving epsilon introns in place. With these mice, we are able to study the changed transcriptional prerequisites in regard to the new germline situation and the resulting effect on the quantity and quality of the IgE response. Indeed, both "Knock-in " mouse strains showed a dramatic increase of membrane IgE transcript if compared to wild type strains. But the more of membrane transcript does not mean the more of transcript for secreted IgE. Increased chimaeric mIgE /IgG1 transcript does not lead to adequately higher transcript for sIgE. This controversial result is also reflected in serum IgE levels of these reporter mice. Summarizing, factors that influence the alternative polyadenylation are largely unknown. However, because expression of mIgE is essential for recruitment of IgE-producing cells in the immune response, clarification of this issue is of great importance. So far, IgE regulation is evident on the level of DNA recombination (switch), transcription and RNA processing. It is not inconceivably that also post-translational and post-transcriptional processes may influence the expression of membrane-bound IgE.

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Insufficient T regulatory cell expression and function in atopic dermatitis skin

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The role of T regulatory (Treg) cells has been widely reported in the suppression of T cell activation. A dysfunction in CD4^{+}CD25^{+} Treg cell-specific transcription factor FoxP3 leads to immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, often associated with atopic dermatitis. Accordingly, increasing the number and activity of Treg cells in affected organs has been suggested as a remedy in various allergic and inflammatory diseases. By immunohictochemistry and lesional T cell purification, we observed that human type 1 Treg cells, their suppressive cytokines, IL-10 and TGF-β as well as receptors for these cytokines were significantly expressed, however CD4^{+}CD25^{+}FoxP3^{+} Treg cells were not found in atopic dermatitis and atopy patch test skin. Molecular pathways of IL-10- and TGF-β-mediated direct T cell suppression were further investigated to better understand why IL-10 and TGF-β cannot suppress skin T cells despite high levels of expression. Here, we show that IL-10 suppresses T cells by blocking CD2, CD28 and inducible co-stimulator (ICOS) co-stimulatory signals, whereas TGF-β additionally suppresses T cell receptor-mediated signals. T cell suppression by IL-10 and TGF-β can be both hindered by high dose superantigens as well as toll-like receptor triggering microbial products. In a rapid signal transduction cascade, IL-10 receptor-associated tyrosine kinase, Tyk2 acts as a constitutive reservoir for the tyrosine phosphotase, SHP-1 in resting T cells. Upon IL-10 stimulation, SHP-1 binds to CD2, CD28 and ICOS co-stimulatory receptors and dephosphorylates them within minutes. In consequence, the binding of phosphatidylinositol 3-kinase to these co-stimulatory receptors no longer occurs and downstream signaling is inhibited. Supporting these data, spleen T cells from SHP-1-deficient mice showed increased proliferation by stimulation via CD2, CD28 and ICOS in comparison to wild-type mice, which was not suppressed by IL-10. Generation of dominant negative SHP-1-overexpressing T cells or silencing of the SHP-1 gene by small inhibitory RNA both abolished the suppressive effect of IL-10 due to altered SHP-1 functions. Genetical alterations in these molecular pathways remain to be elucidated. In conclusion, these data demonstrate a dysregulation of disease-causing effector T cells, in association with an impaired infiltration and function of two major Treg cell subsets.

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Diminished Stimulatory Capacity of Dendritic Cells as a Result of Tryptophan Depletion

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Indoleamine 2,3-dioxygenase (IDO) is expressed by some antigen-presenting cells upon certain stimuli and can degrade the essential amino acid tryptophan. Recent studies show that allergic asthma can be abrogated by IDO-expressing epithelial cells in the lung. IDO is thought to regulate immune responses via tolerogenic effects on T cells, mediated by tryptophan depletion. The data presented here demonstrate that IDO-activity may also affect the T cell-stimulatory

capacity of dendritic cells (DC). Flow cytometric analyses show that monocyte-derived dendritic cells (mo-DC) generated in the absence of tryptophan are altered in their expression of surface antigens related to antigen presentation and costimulatory activities. Specifically, the surface levels of MHC class II molecules, CD80 and CD40 are lower on the surface of mo-DC generated in the absence of tryptophan. Importantly, in mixed lymphocyte cultures (MLRs) mo-DC generated in culture medium without tryptophan displayed a diminished ability to stimulate T cells. In conclusion, a metabolic environment due to ongoing IDO activity such as in allergic inflammation is likely fundamentally to alter APC biology and function. IDO may therefore exert part of its immunomodulatory effect by reducing T cell function at the level of DC.

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Newly produced immature B cells increase in the lung after airway allergen exposure

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Increasing evidence support the role of bone marrow (BM) in allergic airway inflammation. Although B-cell lymphopoiesis in adult mice occurs in the BM, the role of the BM during allergic inflammation remains unclear. Our aim was to determine the B-cell lymphopoiesis in BM and lung respectively 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 to label newly produced cells.BM and lung tissue were taken 24 hours after the final allergen exposure. B220 (B cells), AA4.1 (immature) and BrdU positive cells were determined by Flow Cytometry.

Results: BM $B220^+$ cells were significantly reduced in the OVA compared to PBS exposed mice (7.84±1.61 vs. 17.27±1.2 % of total cells; $p<0.05$). By contrast, there was a significant increase of newly produced B-cells in the BM (91.87±1.45 vs. 86.5±0.78, $p<0.05$; $B220^+/BrdU^+$ expressed as % of total $B220^+$), but no difference in the percent of immature cells (13.72±1.8 vs. 15.39±0.38; $B220^+/BrdU^+/AA4.1^+$ expressed as % of total $B220^+/BrdU^+$).

In the lung there was a significant increase of total cell numbers in the OVA compared to PBS exposed mice (62.75±5 vs. 9.6±2.2 x 10^6; $p<0.05$). Furthermore, there was a significant increase in total number of $B220^+$ cells in the OVA compared to PBS exposed mice (7.13±0.67 vs. 1.26±0.36 x 10^6; $p<0.05$). We also observed a significant increase in the relative number of newly produced cells (18.53±1.76 vs. 9.45±0.68, $p<0.05$; $B220^+/BrdU^+$ expressed as % of total $B220^+$) as well in the relative number of immature cells (32.35±5.48 vs. 18.53±1.76; $p<0.05$; $B220^+/BrdU^+/AA4.1^+$ expressed as % of total $B220^+/BrdU^+$).

Conclusion: Although, allergen exposure decrease the number of $B220^+$ cells in BM, we found an increase in the number of newly produced B lymphocytes, without differences in the degree of maturation. Importantly there was an increase in the number of both newly produced and immature B-cells in the lung after allergen exposure, implying local proliferation of B-cells, or rapid traffic of new and immature B-cells to the lung.

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Costimulation via CD2 upregulates regulatory T cell associated Foxp3

Malin Fagerås Böttcher, Camilla Janefjord, Maria Jenmalm

Statement: Activation via the T-cell costimulatory molecule CD2 enhances antigen recognition and regulates activation of T-cells. A role of CD2 has been implicated in the induction of T-cell anergy and costimulation via CD2 alone, *i.e.* in the absence of costimulation of *e.g.* CD28, has been demonstrated to induce differentiation of suppressive regulatory T cells. Provided that CD2 signalling is important for the control of peripheral T-cell tolerance, a defect CD2 function may disrupt immunological homeostasis. Impaired CD2 function has been associated with development of allergy.

Aim: to investigate the role of CD2 on differentiation of immune responses analysed as cytokine secretion and expression of transcriptional factors and cytokine receptors associated with Th1 and Th2 type immunity and regulatory T-cells in relation to atopic disease.

Material and Methods: Peripheral blood mononuclear cells from 10 allergic and 10 non-allergic adults were cultured in medium alone, in the presence of phytohaemagglutinin (PHA), activating antibodies against CD2 alone and together with antibodies against CD28. Secretion of IL-5, IL-10 and IFN-γ was analysed with ELISA and expression of Foxp3, T-bet, GATA-3, IL-12Rβ2 chain and WSX-1 was analysed with real time PCR.

Results: The expression of regulatory T-cell associated Foxp3 mRNA and secretion of immunosuppressive IL-10 was up-regulated after CD2 activation alone similarly in allergic and non-allergic individuals. Interestingly, after CD2 and CD28 costimulation, the Foxp3 mRNA expression was downregulated. All other parameters were upregulated in both allergic and non-allergic individuals to a similar degree after costimulation via CD2 and CD28 together or after PHA stimulation, whereas no upregulation was observed after CD2 activation alone.

Conclusion: Our study confirms the importance of the costimulatory role of CD2 in the induction of tolerance through the differentiation of regulatory T-cells. We could not confirm earlier reported associations between reduced CD2 function and allergy. Most previous studies have been performed in children, often infants. It is possible that a reduced function of CD2 early in life may affect the derivation of the primary immune responses and is crucial for the clinical outcome, but that the differences regarding CD2 function disappear with age while the allergic phenotype remains.

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Regulatory natural killer cells suppress antigen-specific T cell responses

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The immune system has a variety of regulatory/suppressive processes, which define the equilibrium between healthy and allergic response to environmental antigens. Natural killer (NK)1 and NK2 subsets have been demonstrated to display counter-regulatory and provocative roles in allergic immune responses, similar to Th1 and Th2 cells. Although T regulatory cells that suppress both Th1 and Th2 responses have been the focus of intensive research during the last decade, regulatory subsets of NK cells have not been reported so far. To investigate the existence of regulatory NK cells in humans, NK cell subsets were characterized according to their IL-10 secretion and IL-10-secreting and IL-10-non-secreting NK cells were purified by magnet-activated cell sorting. IL-10-secreting NK cells expressed CD16 and CD56, activation markers CD25, CD69, CD49d, CD45RA, CD45RO, and killer activatory and killer inhibitory receptors CD94, CD1581, CD161, NKAT1, NKB1 on their surface. NK cells showed up to 4 fold increase in IL-10 mRNA by PHA and IL-2 stimulation. In contrast, stimuli which were shown to simulate IL-10 in T cells, such as dexamethasone and/or vitamine D3 did not induce IL-10 mRNA in NK cells. Frequency of IL-10-secreting NK cells was significantly low (2-6 %) compared to IFN-γ-secreting NK cells (61-89 %). As previously observed in $IFN\text{-}\gamma^+$ and $IFN\text{-}\gamma^-$ NK subsets, $IL\text{-}10^+$ and $IL\text{-}10^-$ NK cells did not show any difference in their natural cytotoxicity to K562 cells. The effect of $IL\text{-}10^+$ NK cells on antigen-specific T cell proliferation was examined in bee venom major allergen, phospholipase A_2- or purified protein derivative of *M. bovis*-induced T proliferation. $IL\text{-}10^+$ NK cells significantly suppressed both allergen/antigen-induced T cell proliferation, particularly due to secreted IL-10. For comparison IFN-γ-secreting NK cells did not show any suppression. These results demonstrate that a small fraction of NK cells display regulatory functions similar to T regulatory cells in humans.

Although the suppression of the self-reactive T cells and allergen-specific T cells by T regulatory (T_{Reg}) cells Subsets of T regulatory cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymic selected $CD4^+CD25^+FoxP3^+$ T_{Reg} cells and the inducible type 1 T_{Reg} cells (Tr1). Effector (allergen-specific Th2) and suppressor (allergen-specific T_{Reg}) T cells exist in both healthy and allergic individuals in certain amounts Ηυμαν NK χελλσ ισολατεδ αχχορδινγ το τηειρ ΙΦΝ–γ secretion display distinct cytokine patterns similar to Th1 and Th2 cells.

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Immuno-modulatory effects of viral toll-like receptor-ligands: An experimental approach to the hygiene hypothesis

Running title: effects of viral TLR-ligands on allergic asthma

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Rationale: Epidemiological data suggest that lack of TH1-inducing factors may lead to development of TH2-driven allergic diseases. The role of viral TLR-ligands within this process remains to be completely unclear. **Objectives:** Here we evaluated the impact of activating toll like receptor-3 (TLR-3) or -7 which recognize viral dsRNA or ssRNA on allergic sensitizaton and experimental asthma. **Methods:** Interleukin-12p35 -/- and wild type BALB/c mice were sensitized to ovalbumin (OVA) and allergic airway inflammation was induced by OVA aerosol challenge. TLR-3 or TLR-7 ligands were applied during the sensitization phase or during OVA aerosol rechallenge. **Measurements:** Airway inflammation, BAL cytokine levels and airway reactivity to methacholine were assessed. **Main results:** Systemic application of synthetic TLR-3 or TLR-7 ligands poly (I:C) or R-848, respectively, during the sensitization phase prevented the production of OVA specific IgE/IgG1 antibodies, and subsequently the phenotype of experimental asthma with airway hyperresponsiveness, airway inflammation and increased mucus production. Furthermore, administration of poly (I:C) or R-848 to animals with already established primary inflammatory responses revealed a markedly reduced secondary response following allergen aerosol re-challenges. In contrast to wild type animals, application of poly (I:C) or R-848 to IL-12p35-/- mice had no effect on airway inflammation or goblet cell hyperplasia and AHR. However, even in the absence of IL-12 still a strong suppression in airway eosinophilia and lymphocytes could be observed. **Conclusions:** These data indicate that TLR-3 or TLR-7 activation by viral TLR-ligands has both preventive as well as suppressive effects on experimental asthma which is in part linked to the production of IL-12.

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Peanut specific cytokine responses in non-allergic humans are dominated by T cell-dependent Th2 in the absence of detectable Th1 expression.

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Food allergies, particularly to peanut, are major causes of anaphylaxis. Immune regulation of responses to food antigens is poorly understood. Understanding why sensitization does not usually progress to clinical sensitivity would provide important insight into potential prevention and/or therapeutic options. However, virtually nothing is known of peanut-specific responses in humans who eat peanut without allergic reactions. Most studies focus exclusively on serologic responses and peanut allergic individuals. Here, we developed systems to examine human T cell-dependent responses to peanut allergen in primary culture, directly ex vivo. We investigated the prevalence and nature of cytokine and chemokine responses to this antigen, the co-stimulatory requirements for T cell activation, in peanut exposed non-allergic individuals.

Methods: We recruited 74 adults with no history of peanut allergy, and subsequently divided them into cohorts of history negative/skin test negative vs history negative/peanut skin test positive. Fresh PBMC were stimulated with raw peanut extract in 5d short term primary culture. Resulting type 1 (IFN (, CXCL10) and type 2 (IL-5,IL-13,CCL17,CCL22) responses were evaluated by ultrasensitive ELISAs we developed. Antibodies blocking key coreceptor (CD4, MHC II) or costimulatory (CD28:CD80/86) molecules were used to evaluate activation requirements.

Results: Peanut-specific T cell-dependent type 2 responses, in the absence of detectable Th1 expression, were frequently found in individuals who can ingest peanut without developing symptoms (IL-5,IL-13,CCL17, $p<0.0001$; IFN (, CXCL10, $p>0.05$). History negative/skin test positive adults exhibited higher incidence type 2 responses than did skin test negative (90% vs ~25%, $p=0.002$) . These are CD4 and MHC Class II dependent. They are blocked by CTLA4-Ig or anti-CD86, and less so anti-CD80, but not control IgG.

Conclusion: A substantial proportion of adults who eat peanut without exhibiting symptoms of allergic reactions mount responses to this antigen, regardless of the presence or absence of skin test positivity to peanut. In contrast to recent literature, clinical tolerance did not associate with dominant Th1 activation. The continuum of responses between clinically tolerant, skin test negative, skin test positive and clinically sensitive individuals may be important for development or progression of peanut allergy.

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Interleukin-4 regulates the expression of Thymus- and Activation - Regulated Chemokine/CCL17 by a Signal Transducer and Activator of Transcription 6-dependent mechanism

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The CC-chemokine Thymus - and Activation - Regulated Chemokine (TARC)/CCL17 is considered to be a key mediator in the maintenance of allergic diseases. TARC/CCL17 is constitutively expressed in the thymus and is inducible in several cell-types such as peripheral blood mononuclear cells (PBMCs), macrophages, endothelial cells, epithelial cells, keratinocytes and dendritic cells. Once secreted, TARC/CCL17 selectively binds the chemokine receptor CCR4, which is preferentially expressed on Th2 cells. This receptor-ligand interaction allows recruitment of Th2 cells to the sites of allergic inflammation.

In the present study we have investigated the regulation of TARC/CCL17 expression in primary human T cells. We showed that in human T cells TARC/CCL17 transcription and TARC/CCL17 protein expression is stimulated by Interleukin (IL)-4 in a dose- and time-dependent fashion. Mapping of the TARC/CCL17 promoter region revealed the presence of two binding motifs for Signal Transducer and Activator of Transcription (STAT)6 in proximity to the start position. Electrophoretic mobility shift assays (EMSA) demonstrated that STAT6 was able to bind to both motifs. A fragment of the TARC/CCL17 promoter containing both sites was tested in reporter gene assays for IL-4 inducibility. The promoter was inducible in the STAT6 deficient cell line HEK 293 only upon introduction of functional STAT6. Moreover, point mutations in either of the STAT6 binding sites led to a significant loss of cytokine responsiveness, whereas mutation of both STAT6 binding sites resulted in complete abrogation of TARC promoter activity. These data demonstrate collectively, that human T cells serve as a source of TARC/CCL17 when stimulated with IL-4, and, that the transcription factor STAT6 is essentially involved in this process. As TARC/CCL17 is added to the list of STAT6 regulated genes, our study emphasizes the potential of this transcription factor as a therapeutic target in allergic disease. Taken into consideration the Th2 attractant activity of TARC/CCL17, our findings further indicate a regulatory feedback loop for T cell recruitment.

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Plasma cell differentiation and immunoglobulin-secretion induced by interleukin-4 and anti-CD40 differs in cord blood and adult naïve B cells

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Introduction: Cord blood (CB) B cells produce almost no antibodies except the immunoglobulin (Ig)M isotype, indicating immaturity of the cells or the environment they reside in.

Aim: Our aim was to investigate immunoglobulin isotype switch to IgG4 and IgE in naïve IgD^+ B cells in CB in comparison to adult peripheral blood.

Methods: IgD^+ B-cells from CB and adult peripheral blood were stimulated with IL-4 and aCD40 in the presence of irradiated CD32 transfected fibroblasts. After 12 days of stimulation, the lymphocytes were analysed for surface CD86, CD69, CD45, CD38 and CD138 and intracellular IgA, IgG4, IgE and XBP-1 by flow cytometry. XBP-1 mRNA were furthermore analysed by RT-PCR. Secreted IgA, IgE, IgG4 and IgG1+2+3 were measured by ELISA.

Results: After 12 days of stimulation with IL-4+aCD40, CB and adult blood cells both upregulated CD86 and CD45 in comparison to unstimulated cultures. In CB cultures, also the activation marker CD69 was highly upregulated. Intracellular IgE and IgG4 were greatly increased in CB stimulated cells but only to a minor extent in cells from adult blood. The secretion of IgE however, was similar in both groups whereas IgG4 production was highly increased in cells from adults. Spontaneous formation of IgA- and IgG1+2+3-positive cells were found in both groups in unstimulated cultures. Also both groups displayed a low but detectable IgA production whereas IgG were only substantially upregulated in adult blood cells. The plasma cell markers CD38, CD138 and XBP-1 were highly upregulated in stimulated CB cells compared to cells from adults.

Conclusion: The combination of IL-4 and CD40-ligation increased the numbers of IgE- and IgG4-positive cells significantly both in CB and adult peripheral blood. Thus naïve CB B cells can easily be affected to switch to IgE and IgG4, but are more easily further differentiated to become IgE but not IgG4 producing plasma cells compared to adult B cells. These findings indicate that CB and adult B cells have a similar capacity for IgE secretion when appropriately stimulated.

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Development of Treg, Th1 and Th2-like immunity during the first 2 years of life in relation to allergic disease

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Statement

An understanding of the development of the immune system is required in order to find reasons explaining the increasing incidence of allergies in the industrialized world.

The aim of this study was to investigate the development of spontaneous and phytohemagglutinin (PHA)-induced expression of the T regulatory associated Foxp3, the Th2-associated GATA-3 and the Th1-associated T-bet, IL-12R$^2_2$ and WSX-1, in allergic and non-allergic children followed from birth and up to two years of age.

Method

Thirty-four children were followed prospectively during their first two years of life. The allergic status was based on clinical examination, skin prick tests and questionnaires. Venous blood was drawn at birth (cord blood), 3 or 6, 12 and 24 months of age. Peripheral blood mononuclear cells were cultured with 2 ¼g/ml of PHA or with medium alone for 24 h. The mRNA expression of the different markers was analysed with real-time PCR.

Results

The spontaneous expression of the different markers was similar at birth and at 24 months, except for a tendency towards increasing Foxp3 with age.

Stimulation with PHA induced an increased expression of all markers, except GATA-3, at all ages, both in allergic and non-allergic children. GATA-3 was up-regulated at birth in all children but only in allergic children at 6 and 12 months. In contrast, PHA-stimulated T-bet and WSX-1 expression increased from birth to 24 months in the non-allergic, but not in the allergic, children (n=9, p=0.03 and n=8 p=0.01 respectively). For WSX-1, this was probably due to a significantly higher expression at birth in the allergic, as compared to the non-allergic, children (n=15, p=0.045). PHA-induced Foxp3 and IL-12R$^2_2$ expression did not differ between birth and 24 months of age.

Conclusions

The different development of Th1 and Th2- related factors in allergic and non-allergic children may influence the development of allergic disease in childhood.

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Endotoxin-induced cytokines from antigen presenting cells during the first two years of life in Estonian and Swedish children

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Statement: Our aim is to investigate the development of antigen presenting cell function (LPS/IFN-γ-induced cytokine responses) during the first two years of life in two geographically adjacent countries with marked differences in living conditions and incidence of atopic diseases, *i e* Estonia (low incidence) and Sweden (high incidence), in order to understand the impact of the environment.

Methods: The development of LPS/IFN-γ-induced IL-1β, IL-6, IL-10, IL-12p70, MIP-1β and TNF responses were studied from birth up to the age of two years in 14 Estonian and 36 Swedish infants. Clinical examinations and skin prick tests were performed and blood samples obtained at birth and at 3, 6, 12 and 24 months. Blood mononuclear cells were stimulated with LPS and IFN-γ. Cytokines were analysed by Luminex.

Results: LPS/IFN-γ-induced IL-1β, MIP-1β and TNF responses were low at 6 months as compared to the other ages, while IL-10 and IL-12p70 were similar over time. Swedish children showed higher cytokine responses than Estonian children (higher IL-6 and MIP-1b at birth, higher IL-1β, IL-6, IL-12p70 and TNF at 3 months of age and higher IL-6 at 6 months of age). Atopic symptoms and sensitization were associated with higher IL-10 and IL-1β responses at 3 and 12 months of age, high IL-12p70 levels at 3 months of age and high MIP-1β levels at 6 months of age.

Conclusions: The enhanced secretion of proinflammatory cytokines from Swedish, as compared to Estonian, children may be due to a less rapid induction of immune regulation, and mirrors the disparate expression of atopic disease, which subsequently develops in the respective populations.

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Regulation of Dendritic Cell Maturation and Function by Bruton's Tyrosine Kinase via IL-10 and Stat3

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Bruton's Tyrosine Kinase (Btk), a member of the Tec family, plays crucial roles in the differentiation and activation of B and myeloid cells. Mutations in the *btk* gene cause severe defects in adaptive immunity, leading to X-linked agammaglobulinemia in human patients and X-linked immunodeficiency (*xid*) in mice. *Btk* mutations in XLA patients lead to a block in the pro-B to pre-B transition during B cell ontogeny, resulting in a deficit of mature B cells and serum immunoglobulins. *Xid* and *btk*$^{-/-}$ mice have milder phenotypes. B cells from *btk* mutant mice respond abnormally to crosslinking of several cell surface receptors including BCR and some cytokine receptors. Btk's roles have also been shown in Fc receptor-mediated mast cell and myeloid cell activation as well as collagen receptor-mediated platelet functions. Despite drastic reductions of other immunoglobulin isotypes, paradoxically high IgE responses to immunization and parasite infection have been known in *btk* mutant mice for more than 25 years. Here we show that Btk-deficient dendritic cells exhibit a more mature phenotype and a stronger in vitro and in vivo T cell-stimulatory ability, compared to wild-type dendritic cells. Increased IgE responses were induced by adoptive transfer of Btk-deficient dendritic cells into mice. Consistent with the stronger T cell-stimulatory ability of Btk-deficient dendritic cells, Btk-deficient mice exhibited enhanced inflammation in Th2-driven asthma and Th1-driven contact sensitivity experiments. These negative regulatory functions of Btk in dendritic cells appear to be mediated mainly through autocrine secretion of IL-10 and subsequent activation of Stat3. Thus, this study has resolved a long-time enigma of paradoxical IgE responses in *btk* mutant mice and revealed a novel aspect of Btk function in dendritic cells, the most important antigen-presenting cells.

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Peroxisome-proliferator activated receptor gamma- and Toll-like receptor-2 agonists might have therapeutical value in Respiratory syncytial virus (RSV) induced airway disease

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Respiratory syncytial virus (RSV) is worldwide the leading cause for severe lower respiratory tract infection (LRTI) in infants requiring hospitalization. Evidence accumulated that RSV-induced LRTI leads to bronchiolitis, pneumonia, airway hyperresponsiveness (AHR) and perhaps asthma. Persistent lung function abnormalities were even observed 20 years following RSV infection. Family histories of asthma and atopy are well accepted risk factors for RSV-associated AHR. However, currently, there exist neither an effective active vaccine nor a promising antiviral or anti-inflammatory therapy. All three issues (i) the viral lytic replication process, (ii) the fulminant cytopathic effect primarily mediated by the viral fusion protein (F protein), (iii) and the RSV-induced intense inflammatory lung response are considered to be responsible for the detrimental outcome of RSV infection. Quite recently, we presented data showing that the addition of specific peroxisome-proliferator activated receptor-γ (PPARγ) agonists (15d-PGJ$_2$, Fmoc-Leu, ciglitazone, and troglitazone) to RSV-infected human lung epitehelial cells (A549, NHBE) led to a significantly reduced cell surface expression of ICAM-1- and MHC-I molecules. Furthermore, the release of immunomodulatory (IL-6, GM-CSF) and proinflammatory cytokines (IL-1α, TNF-α) as well as chemokines (IL-8, RANTES) was drastically reduced. A down-regulated binding activity of NF-κB (p65/p50) and AP-1 (c-fos) paralleled these findings. Since RSV replication leads to a fulminant activation of NF-κB in epithelial cells, we, therefore, asked whether the replication of RSV might be directly influenced by activation of PPARγ. Our data show that activation of PPARγ, but not PPARα,

inhibited by nearly 1000fold the replication of RSV in lung epithelial cells. Concomitantly, the expression of the viral G- and F protein was significantly reduced. Confluent RSV-infected A549-monolayers were fully protected from cytopathic cell damage. In addition, we observed an RSV-dependent upregulation of toll-like receptor (TLR)-2 on A549 cells. But in contrast to the cell mediators mentioned above the activation of PPARγ had no impact on RSV-induced TLR-2 expression. However, specific activation of TLR-2 reduced the RSV-induced expression of ICAM-1 suggesting an anti-inflammatory potential. Our data suggest that specific stimulation of PPARγ and TLR-2 in RSV-infected human lung epithelial cells might have beneficial effects in the control of RSV-induced lung disease.

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Molecular Mechanism of Th1û0Th2 Imbalance and Hygiene Hypothesis

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Allergic disorders are developed by combination of genetic risk factors and environmental factors. Th1 and Th2 imbalance is one of important mechanisms for development of allergic disorders. The occurrence of autoimmune diseases and allergic diseases became higher in more affluent, Western, industrialized countries. One theory proposed to explain the increase in the prevalence of autoimmune and allergic diseases is that it results from a decrease in the prevalence of childhood infection. This theory dates back to the mid-1960s in relation to Th1-mediated diseases. In 1989, Strachan first proposed that this theory might also explain the increase in Th2-mediated diseases, and it has subsequently come to be called the hygiene hypothesis. Here, we present the molecular mechanism of the Th1 and Th2 system and the hygiene hypothesis.

Atopy is characterized by enhanced IgE responses. The production of IgE is upregulated by Th2 cytokines, in particular, IL -4, and is downregulated by Th1 cytokines, in particular, IFN -³. IL-12 and IL-18, which produce IFN-³, are the important cytokines that downregulate IgE production. Imbalance in Th1 and Th2 system induces the allergic diseases. We show genetic and gene expression (RNA processing) defects in the downregulation of IgE production, especially mutations of the IL-12 receptor ²2 chain gene and the IL-18 receptor ± chain gene in IL-12 and IL-18 signalings, in atopy.

CD25-positive T cells and other regulatory T cells produce IL-10 and TGF-², and act to downregulate both Th1 and Th2-mediated responses. So, we investigated the effects of IL-10 on Th1 and Th2 cytokine productions, and IL-10 production. IL-10 suppresses accelerated production of both Th1 and Th2 cytokines, so, IL-10 acts as an immunomodulator and keep the Th1û0Th2 balance. IL-10 production from PBMCs stimulated with lipopolysaccharide0on allergic patients was significantly lower than that on healthy controls, showing that, at least, in allergic patients, the development of IL-10 production is poor. This may result from poor development of regulatory T cell and so on. As a result, Th1û0Th2 imbalance may be raised.

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Monocyte response to LPS and LTA in allergy

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Background: Allergy has increased in the western world and an altered microbial exposure has been suggested as a possible explanation. Worldwide, ongoing studies evaluate the allergy preventing effect of probiotic bacteria. The responses to microbial components are influenced by genetic factors, however. We have previously shown that the AG genotype of the TLR4 Asp299Gly polymorphism is associated with decreased lipopolysacharide (LPS) induced cytokine secretion and atopic asthma. The interactions between genetics, bacterial induced immune responses and development of disease must be further elucidated for evaluation of the potential of probiotics in prevention and treatment of allergy.

Aim: To study bacterial lipoteichoic acid (LTA) and LPS induced immune responses in relation to atopic disease.

Material and Methods: Three individuals with asthma, 4 with allergic rhinoconjuncitivitis (ARC), 9 with both asthma and ARC and 27 healthy young adults with no allergy were included. DNA was sequenced regarding the TLR4 Asp299Gly polymorphism. Eight individuals had the AG genotype while the others had the wildtype, AA. Whole blood and peripheral blood mononuclear cells (PBMC) were stimulated with bacterial LPS or LTA for 16h. Cells in the whole blood cultures were analysed with flow cytometry regarding monocyte activation and functional receptors. Cell supernatants from the PBMC cultures were analysed for cytokines with Luminex technique.

Results: Percentage of TLR2 expressing CD14+ cells in fresh whole blood was higher among AG individuals compared to AA. The median flourescence intensity (MFI) of TLR2 increased after LPS and LTA stimulation. The increase in TLR2 MFI after LTA stimulation was higher among the healthy individuals compared to those with allergy. The MFI of CD80 expression on CD14+ cells after LTA stimulation increased in the non-allergic but tended to decrease in the allergic individuals.

The IL12(p70) and IL-17 secretion was higher after LTA stimulation in the allergic, as compared to the healthy, group.

Conclusion: The TLR4 polymorphism affects the innate immune response induced by microbial products. The immune response to LTA differs between allergic and healthy individuals, which may be associated with the susceptibility to allergies.

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Long-lived plasma cells and their contribution to allergen-specific IgE

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IgE antibodies play a major role in the pathogenesis of typ I allergies. As the half life of serum IgE is short, plasma cells continuously have to secrete large amounts of IgE to maintain the serum titers over long periods of time. It is currently debated, whether IgE-secreting plasma cells are short-lived end products of a chronic activation of B cells, or long-lived, if maintained in supportive niches of the bone marrow or in inflamed tissues. We have analysed proliferation and lifetime of IgE-secreting plasma cells in a murine ovalbumin (OVA) allergy model. The time point of origin and the plasma cell turnover in the OVA allergic mice were determined according to incorporation of BrdU into DNA of proliferating cells. Organs (spleen, lung, lymph nodes, bone marrow) and sera were analysed using ELISPOT, ELISA, fluorescence microscopy and flow cytometry. 4-6 weeks old mice were sensitized with OVA and then continuously fed BrdU for 2 weeks, supplied via their drinking water. 25% of IgE-secreting plasma cells in spleens of the OVA allergic mice were BrdU-positive, indicating that they had proliferated within the time of BrdU-feeding. 75% of the IgE-secreting splenic plasma cells had been generated before that time period and thus had a lifetime of more than 2 weeks. Anti-proliferative, immunosuppressive therapy (cyclophosphamid) did not eliminate the cells producing OVA-specific IgE antibodies, indicating that the respective plasma cells are not dividing and long-lived. IgE-secreting plasma cells can be long-lived. These long-lived, IgE-secreting plasma cells provide allergen-specific IgE independent of the presence of allergen and are resistant to immuno suppression.

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Assessment of allergen-specific T cell responses in humans with HLA-class II peptides tetramer technology

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Rationale: Whereas allergic patients develop allergen-specific Th2 responses, there is some evidence that non allergic persons exposed to allergens rather mount protective T cells responses skewed towards regulatory T cells. Also, successful immunotherapy appears to induce $CD4^+$ $CD25^+$ or Tr1 regulatory T cells in allergic patients. In this context, sensitive tools need to be developed to assess in details allergen-specific T cell responses in allergic patients during immunotherapy. Also, such tools would allow to better characterize T cell responses in non allergic people, in order to identify correlates of immune protection, facilitating the design of optimized vaccines.

Methods: To design HLA-class II-peptide tetramers, T cell epitopes from the birch pollen allergen Bet v1 were identified using computer algorithms, and peptide binding experiments were performed. The highest affinity peptides were combined with recombinant HLA-DR molecules labelled and polymerized with streptavidin/biotin. Multimeric (tetrameric) high avidity complexes thus obtained and cytofluorometry are used to detect and isolate allergen-specific T cells from peripheral blood, which can be further characterized.

Results: We have developed Bet v 1 specific HLA class II tetramers to assess T cell responses in 40% of the Caucasian population. To this aim a library of peptides covering the entire Bet v1 sequence has been synthesized and tested *in vitro* for binding to major HLA haplotypes. Five T cell epitopes have been identified within Bet v1, including the major immunodominant epitope (peptide 140-153). The latter has been combined with recombinant HLA DR B1 0101, 0401, 1501 molecules to form tetramers. The HLA-DR B1 1501-peptide tetrameric complex labelled with a fluorescent dye was used as a probe to detect Bet v 1-specific $CD4^+$ T lymphocytes in the peripheral blood of an allergic patient with the proper HLA haplotype. Interestingly, we could also detect numerous Bet v1-specific T cells in two healthy individuals. Using cell sorting and immunopurification with magnetic beads, HLA-tetramer positive cells have been isolated from all samples and clones are being derived. These T lymphocytes will be assessed at a single cell level for cytokine production, T cell receptor Vß usage and gene expression.

Conclusion: We have developed a powerful tool to compare allergen-specific T cell responses in both allergic and non allergic human beings, as well as to monitor immunotherapy. The detailed characterization of allergen reactive T cells obtained from healthy donors should lead to the identification of immunological correlates of protection.

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T cell regulation of neutrophilic inflammation

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Acute generalized exanthematous pustulosis (AGEP) is a peculiar drug hypersensitivity reaction, which was formerly considered as pustular psoriasis of Zumbusch type, but is now recognized as an own entity, which is mainly elicited by drugs: Within 3-4 days after start of drug treatment >100 sterile pustules appear on the skin, and the patient has fever and leukocytosis. Stop of treatment leads rapidly to disappearance of the pustules. Immunohistochemistry as well as analysis of drug specific T-cell clones derived from blood or affected skin suggested that T-cells regulate this sterile, polymorphonuclear neutrophil (PMN) rich skin inflammation: First drug specific T-cells with cytotoxic potential (perforin and FasL +) infiltrate the epidermis where they form vesicles, later drug specific T-cells secreting high levels of CXCL8 contribute to the recruitment of PMN, which transform the vesicles into pustules. The T-cell clone derived supernatants acts anti-apoptotic and chemotactic for PMN, which is partly blocked by anti CXCL8 antibodies.

We recently analyzed also Patients with auto-inflammatory diseases like pustular psoriasis and Behçet's disease, where neutrophilic inflammations do also occur. Again such peculiar, CXCL8 producing T-cells were detected using both immunohistochemistry and cloning of T cells from skin biopsies: A pronounced infiltration of PMNs and of CD4+ T-cells was found in skin biopsies from pustules obtained from patients with pustular psoriasis and Behçet's disease, with the infiltrating T-cells strongly positive for CXCL8 and the chemokine receptor CCR6, which characterizes skin infiltrating, inflammatory T cells. The T cell clones obtained from these skin biopsies from patients with pustular psoriasis or Behçet's disease produced high amounts of CXCL8 and GM-CSF, often together with IFN-γ and TNF-α. Interestingly, some T cell clones failed to secrete IL-5 and/or IFN-γ and did thus not correspond to Th1 or Th2 T-cells and may represent an own T cell subset organizing neutrophilic inflammations.

In conclusion, our data from AGEP as well as other sterile pustular skin diseases revealed the presence of peculiar T cells able to recruit PMN and thus contributing to sterile, neutrophilic diseases.

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Free immunoglobulin light chains (FLC) in hypersensitivity reactions: need for crosslinking by antigens

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Background:
Recently, we have shown that FLC can play a crucial role in the induction of contact sensitivity and non-atopic asthma. Our current working model proposes that antigen-specific FLC sensitize mast cells through specific receptors on the cell surface. Following a second contact with the cognate antigen, surface-bound FLC are cross-linked leading to mast cell activation. In current study, we investigated whether cross-linking of FLC is indeed necessary to induce hypersensitivity responses.

Experimental methods:

Passive cutaneous anaphylaxis (PCA):

PCA1: Mice were intradermally sensitized with dinitrophenol(DNP)-specific FLC or IgE. Twenty hours after sensitization, mice were intravenously challenged with multivalent antigen (DNP-human serum albumin (35:1)) and ear swelling responses were measured. In a second group, mice were challenged in presence of a molar excess of monovalent antigen (DNP-alanine).

PCA2: Mice were intradermally sensitized with two different Der p 2-specific FLC or with either one of the FLC preparations alone. Twenty hours later, mice were systemically challenged with recDer p2 or house dust mite extract and ear swelling responses were measured.

Results:
PCA1: Mice sensitized with DNP-specific FLC and systemically challenged with DNP-HSA develop a rapid ear swelling response with maximal swelling at 30 min after challenge. When the sensitized mice were challenged with DNP-HSA in combination with monovalent hapten DNP-alanine, no significant swelling was observed. Also no ear swelling was present in mice challenged with DNP-alanine only. Similar results were obtained in the IgE-sensitized animals.

PCA2. Passive sensitization of mice with the two different Der p 2-specific FLC followed by challenge with house dust mite extract or recDer p 2 resulted in a rapid development of ear swelling. Both FLC were necessary to elicit this ear swelling response, because sensitization with either of the Der p 2-specific FLC alone did not result in a ear swelling response after challenge.

Conclusions:
In presence of a molar excess of monovalent antigen, no crosslinking is expected and no swelling was induced. Elicitation of ear swelling by Der p 2 needed the presence of at least two FLC directed to different epitopes on this allergen. In conclusion, these experiments clearly demonstrate that crosslinking of FLC is necessary to induce cutaneous ear swelling responses.

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Macrophage-derived interleukin-10 controls the inflammatory response mediated by TLR-4 but not the response to TLR-9 ligation

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Interleukin-10 (IL-10) is an important regulator of immune responses secreted by a variety of cell types including macrophages, lymphocytes, epithelial and mast cells. While complete IL-10 deficiency in IL-10-/- mice results in exaggerated T cell as well as innate responses, we have previously shown that mice with a selective inactivation of the IL-10 gene in T cells suffer from deregulated T cell responses exactly as in $IL\text{-}10^{-/-}$ animals. In contrast to $IL\text{-}10^{-/-}$ mice, the T cell-specific mutants do not mount enhanced innate responses, which therefore must be subject to control by IL-10 from non-T cells. We now generated mice with a cell type-specific IL-10-deficiency in macrophages and studied the local and systemic inflammatory response to CpG-oligonucleotides and LPS mediated by TLR-9 and TLR-4, respectively, in these mutants. Subcutanous injection of both TLR ligands in wt mice results in a moderate local inflammatory infiltration dominated by macrophages. The macrophage-specific IL-10 mutants developed an enhanced inflammatory infiltration as well as extensive tissue necrosis. The mutants also showed increased serum levels of proinflammatory mediators after intraperitoneal LPS injection in comparison to wt mice. In contrast, the local and systemic inflammatory responses of macrophage-specific IL-10 mutants to CpG-oligonucleotides were indistinguishable from CpG responses in wt mice. These results show that different innate responses can be subject to control by IL-10 from different cellular sources. We are presently working on the identification of the IL-10-secreting cell type responsible for regulation of the CpG response. In addition, we are investigating innate responses in cell type-specific IL-10 receptor mutants.

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M-DC8+ blood dendritic cells are principal producers of early interleukin-12 and are tightly controlled by contact with erythrocytes

Knut Schäkel, Matthias von Kietzell, Annette Ebling, Livia Schulze, Michael Haase, Christian Semmler, Marika Sarfati, Gwendalyn Randolph, Michael Meurer, Ernst Peter Rieber

High level production of interleukin 12 (IL-12) is crucial for effective immune responses against pathogens and its relevance in chronic allergic inflammation is increasingly recognized. However, the nature of cells producing early and high levels of IL-12 remain poorly defined. Here we show that a subset of human blood dendritic cells (DCs) which is characterized by a carbohydrate modification of the PSGL-1 and recognized by the mAb M-DC8 is the principal and primary source of IL-12p70 when blood leukocytes are stimulated with the Toll-like receptor 4-ligand lipopolysaccharide (LPS) or with CD40L. To respond to LPS these DCs require a short phase of in vitro maturation which is completely blocked in the presence of erythrocytes. This maturation depends on the interaction of CD47 on erythrocytes and its ligand signal-regulatory protein α (SIRP-α) on M-DC8$^+$ DCs. While tightly controlled in blood by erythrocytes, in tissues the high IL-12- and TNF-α-producing capacity of M-DC8$^+$ DCs may be critical for the defense against pathogens and, if uncontrolled, may lead to adverse inflammatory reactions. Accordingly, we identified M-DC8$^+$ DC within the dermal inflammatory infiltrate of atopic dermatitis and psoriasis vulgaris.

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Reduced allergic airway inflammation and lack of airway hyperresponsiveness (AHR) following sensitisation with dendritic cells over-expressing IL-10.

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Background: Lung dendritic cells (DC) are essential for presentation of allergens to T-cells in primary airway sensitisation and during allergen challenge via the airways. IL-10 is thought to have a critical role in the development of regulatory T-cell responses. Here, we study effects of local IL-10 over-expression in genetically modified DC on allergic airway sensitisation.

Methods: Bone marrow derived DC (bmDC) were generated in culture with GM-CSF and transduced with IL-10 expressing lentiviral vectors. Transduced bmDC (IL-10 OVA-bmDC) and controls (OVA-bmDC) were pulsed with ovalbumin (OVA) and transferred intratracheally to naïve BALB/c mice. Mice were then challenged intranasally with OVA 10, 11 and 12 days after transfer. 24hrs after challenges, airway responsiveness to methacholine was assessed by barometric whole body plethysmography and airway cells were analysed by differential cell count. Further, DC-induced proliferation of OVA-specific DO11.10 CD4$^+$T-cells was studied in vitro.

Results: Transfer of OVA-bmDC followed by OVA challenge resulted in AHR (maximal Penh 10.3±1.2 versus bm-DC 3.3±0.6, $p<0.01$) and airway eosinophilia (from 0.17±0.06% to 37.3±1.5%, $p<0.01$). In contrast, challenge following transfer of IL-10-OVA-bmDC did not result in AHR (maximal Penh 4.3±1.4) and induced significantly less eosinophilia (11.6±0.9%, $p<0.01$). IL-10 levels in the airways following transfer of IL-10-bmDC did only increase marginally, while large amounts of IL-10 were produced in vitro by IL-10-bmDC. In vitro, IL-10-OVA-bmDC induced significantly less proliferation of DO11.10 CD4$^+$T-cells than OVA-mbDC, but antigen-specific T-cell proliferation was not abrogated. CD4$^+$T-cells from co-culture with IL-10 OVA-bmDC, but not with OVA-bmDC partially inhibited CD4$^+$T-cell proliferation if added to a secondary co-culture.

Conclusions: IL-10-OVA-bmDC do not induce adequate sensitisation and thus do not lead to AHR and only to weak allergic inflammation, although they are capable of inducing OVA-specific T cell proliferation. Our in vitro data suggest that IL-10-OVA-bmDC induce regulatory CD4$^+$ T cells, which may limit allergic airway inflammation in vivo.

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Dermal fibroblast potently induce maturation of dendritic cells (DC)

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To trigger effective T-cell-mediated immune response DC have to migrate from peripheral tissues such as the epidermis, via the dermis into locally draining lymph nodes (LN) where they present antigen to naive T cells. During this migration DC undergo distinct phenotypic and functional changes termed collectively *DC maturation*, which can be recapitulated during in vitro DC culture. To date little is known about the signals delivered to DC by the various cellular microenvironments that DC encounter during their travel from peripheral epithelia to lymphoid tissues. In a first attemt to address this issue we studied the interaction of DC with fibroblasts the major cellular component of the dermal microenvironment. Here we report that in vitro human DC have the capacity to adhere specifically to human dermal fibroblasts via the interaction of the β2-integrins (on DC) and Thy-1 (CD90) and ICAM-1 (on fibroblasts). Moreover, in the dermis of an evolving cutaneous immune response such as allergic contact dermatitis, β2-integrin-positive DC are found in close apposition to Thy-1/ICAM-1-positive fibroblasts. To study the effects of such contacts on DC immune functions, human DC were generated from CD14+ PBMC using GM-CSF and IL-4 containing media and were co-cultured for 24h with allogeneic or autologous fibroblasts. .By flow cytometry we showed that many maturation markers among them CD80, CD83, HLA-DR were induced or up-regulated on DC upon co-culture with fibroblasts. Separation of DC and fibroblasts by transwell-inserts revealed that both a direct cell-cell contact as well as soluble mediators are responsible for the fibroblast-induced maturation of DC. Based on preliminary results we suppose that adhesion mediated by β2-integrin (on DC) and Thy-1/ICAM-1 (on fibroblasts) results in a dramatic stimulation of autocrine TNFα secretion by DC, which in turn induces full DC maturation.

In summary we demonstrate that human dermal fibroblasts potently induce differentiation of immature DC to mature DC via β2-integrin/Thy-1/ICAM-1 interactions and the stimulation of an autocrine TNFα loop. Our data are consistent with the notion that the dermal cellular microenvironment actively participates in the regulation of DC immune functions.

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Creating Artificial Allergens and Antibodies for Immunotherapy

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The currently known allergens can be grouped in less than 100 structural sequence motifs by bioinformatic analysis. Cross reacting antibodies against different allergens recognize such motifs. Allergenic structures are a narrower definition than protein families. Thus, the true number of allergens may be smaller than previously assumed. This opens the possibility to use prototypic allergenic structures, generated in the form of consensus sequences, either for diagnostic purposes or for immunotherapy. This would decrease the necessary number of allergens for traditional immunotherapy. We have generated such an artificial tropomyosin allergen based on a consensus sequence within an allergen motive. This artificial protein was equally well recognized as the recombinant full lenght of tropomyosin.

Anti-IgE treatment in the form of omalizumab (Xolair) has been shown to be effective and many other antibodies are being used for therapy of various diseases. However, cost is a disadvantage of most passive immunotherapy. Thus, cheaper artificial antibodies may be used in the future for the therapy of allergic disease. Using designed ankyrin repeat proteins (DARPINs) and ribosomal display it is possible today to mimic functional antibodies. Such artificial antibodies, produced in prokaryontic expression systems, have no disulphate bridges and are extremely stable. Thus, they have advantages over traditional monoclonal antibodies in terms of price, production and storage. We have isolated DARPINs against IgE, antibody binding sites and the FcεRI. The number and the specificity of these binding proteins were greater than usually achieved by antibodies isolated by phage display. DARPINs are ideal tools for in vitro purpose and are presently in preclinical testing.

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NKT cell triggered IL-21 production induced selective Bε cell apoptosis and suppression of IgE responses in mice and humans

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It has been proposed that the increase in prevalence and severity of allergic disease, mediated by IgE, inversely correlates with exposure to infectious diseases and *Mycobacterium bovis* bacillus Calmette Guerin (BCG) vaccination. Here, we demonstrate that Vα14 NKT cells are required for the BCG-mediated IgE suppression. BCG-activated Vα14 NKT cells suppressed an antigen specific IgE and total IgE but not IgG1 and IgG2a responses through their IL-21 production, because IL-21 was produced mainly by Vα14 NKT cells but not by conventional CD4 T cells, which was distinct findings from those published previously. The IL-21 expression of Vα14 NKT cells activated by BCG seemed to require for IL-12 signaling mediated by dendritic cell activation through innate immune signaling pathway including MyD88/IRAK-4. However, BCG-mediated signals were independent of TLR 2 and 4, because TLR2/4-KO DCs were able to provide IL-12 signals to NKT cells, suggesting that other TLRs may involved in the production of IL-12. The addition of BCG-stimulated Vα14 NKT cells or recombinant IL-21 in the Bε cell culture resulted in a dramatic IgE suppression, which was abrogated by addition of anti-IL-21. The specific IgE suppression mediated by IL-21 is due to the specific apoptosis induction on Bε cells but not on Bγ cells. The findings were supported by the augmented expression of Bmf, apoptosis accelerated gene, and also down-modulated expression of anti-apoptotic gene, Bcl-xl, in Bε cells but not Bγ cells. Moreover, IL-21-mediated Bε cell apoptosis was abrogated by anti-IL-21.

IL-21 was also produced by human PBMC, in particular Vα24 NKT cells and conventional CD4T cells, after BCG-vaccination and selectively induced apoptosis of human Bε cells. In fact, when we vaccinated volunteers having cedar pollen pollinosis with BCG, the cedar pollen-specific IgE responses were dramatically decreased. These results provide as yet unknown mechanisms for the therapeutic effect of BCG vaccination, which has been broadly used in various clinical settings.

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Relationship between Human anti-Chimeric antibodies to infliximab in relationship to infusion related allergic reactions in patients with rheumatoid arthritis

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Half of RA patients treated with infliximab develop antibodies to infliximab, (HACAs; Human anti chimeric antibodies). Development of HACAs is related to reduced response to treatment and infusion related allergic reactions. Recently, we found that infusion reactions are associated with the formation of larger immune complexes. Since infusion reactions were not observed in all patients that develop HACAs, we investigated whether the occurrence of infusion reactions is influenced by the relative contribution of IgG4 to the HACA response knowing that IgG4 is unable to form large immune complexes and has been described to be protective against immune complex induced disease.

Methods: From a cohort of 200 RA patients receiving infliximab we identified 19 patients with an infusion reaction. Total IgG and IgG4 levels against infliximab were determined in sera collected prior to the infusion reaction. As internal control, IgG and IgG4 were determined in sera collected from the same patients prior to a previous infusion, not causing any clinical problems. Furthermore, results were compared with sera collected from patients that did develop HACAs but showed no infusion reaction.

Results: Infusion reactions observed in the 19 infliximab treated RA patients were mild. Most reactions occurred early (weeks 6 and 14 during treatment). The reactions consisted of erythema, dyspnoe and increased heart rate, only few patients had a significant drop of blood pressure. In some fever was observed several hours after infusion. All patients recovered within a few hours.

HACA levels were significantly higher in sera collected immediately prior to an infusion reaction (median: 7.9 ¼g/ml [0.46-1917]) compared to the internal control (median: 0.24 ¼g/ml [0.13-225]). The median relative contribution of IgG4 to HACA levels is 29% (1-100%). This is similar to the internal control (median: 33%; 1-100%) and to patients without infusion reaction (median 32%; 7-60%).

Conclusions: Infusion reactions to infliximab are associated with increased HACA levels, however not all patients with HACAs show infusion reactions. This discrepancy cannot be explained by a difference in the relative contribution of IgG4 to HACAs.

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Risk assessment for elderly to develop sensitization to respiratory and food allergens

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Background: For elderly people, epidemiological data on respiratory allergies are rare and for food allergy completely missing.

Objective: The aim of this study was to examine the prevalence and risk factors for sensitizations in 109 people with a mean age of 77 years and resident living in a geriatric nursing home.

Methods: The cross-sectional study included a detailed interview, skin prick and serum tests for specific and total IgE, IFN-³, and ST2, a marker for Th2-lymphocyte activity.

Results: Almost all study subjects (n=101) suffered from co-morbidity, 14 from type I allergy, 25 from gastrointestinal disorders treated with anti-ulcer drugs, 25 were chronic alcoholics and 21 were smokers. The total IgE levels were significantly higher in men (p=0.025), and not affected by smoking or alcohol consumption. Skin prick tests were positive in 36,7 % of tested patients. Specific IgE to respiratory allergens was found in 40,4 % of all patients and was elevated in men (p=0.013), with a significant correlation to smoking (p=0.029). Specific IgE to food allergens was detected in 24,8 %, apparently without connection to the investigated risk factors. However, positive skin prick tests with food allergens could be correlated with chronic alcohol consumption (p=0.036). The intake of anti-ulcer medication was significantly correlated with elevated ST2 levels as an indirect readout for Th2-cell activity (p<0,001).

Conclusion: The risk factors for sensitization in elderly to respiratory allergens were chronic damage of respiratory epithelia due to smoking, and for sensitization to food allergens chronic alcohol consumption.

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A novel function of nerve growth factor: A regulatory role in mucus

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Background

Several molecules have been shown to play a role in mucus production and possibly in diseases characterized by mucus metaplasia and/or accumulation. Nerve Growth Factor (NGF) is a neurotrophin also involved in inflammation and tissue remodelling.

Personal findings

NGF is increased in serum and secretions of several diseases associated with mucus hyperproduction

NGF (and its receptors) are widely expressed in mucosal tissues, particularly by epithelial cells, infiltrating inflammatory cells and in proximity of mucus glands

In experimental animals, epithelial damage causes NGF release

NGF is produced in large amounts by rat submaxillary glands and, in man, by several structural and inflammatory cells including mast cells, eosinophils and CD4-Th2 lymphocytes

NGF transgenic mice show a higher expression of mucin 5AC mRNA and protein vs wild type animals. Mucin 5AC (mRNA and protein) increase after ovalbumin sensitisation and challenge, particularly in transgenic animals

In cell-coltures, NGF influences epithelial differentiation as well as mucin mRNA and protein expression.

Conclusions

Taken together these data indicate a novel function of NGF which should be included in the list of molecules regulating mucus production and involved in diseases with mucus metaplasia and accumulation.

Acknowledgment

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Chronic *Chlamydia pneumoniae* infection promotes atherosclerosis by inducing an IL-4-dependent hypersensitivity reaction

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Objective: Atherosclerosis is an inflammatory response, probably to a range of initiating causes. Chronic infection with *Chlamydia pneumoniae* (C.pn) has been suggested as one cause, but the nature of the association is controversial, in large part due to lack of an identified mechanism to link infection with the atherosclerotic process in man. The aim of the study was to correlate the serological status of C.pn infection with the pattern of secretion of cytokines from CD4+ T lymphocytes.

Method: The host response to C.pn infection in 139 subjects having angiography to investigate stable chest pain was assessed in terms of cytokine secretion using a whole blood culture method, with or without added C.pn antigen. *H.pylori* infection status was used as an infection control.

Results: C.pn seropositive subjects secreted significantly more IL-4 than did those who were seronegative (p=0.02). No significant difference was noted for secreted IFN-γ. The amount of secreted IL-4, but not of secreted IFN-γ, positively correlated with the extent of coronary artery disease, albeit in a non-linear fashion (p=0.006). A similar correlation with secreted IL-4 was not identified with *Helicobacter pylori* infection.

Conclusions: This is the first evidence of a mechanism that links C.pn infection with coronary artery atherosclerosis. IL-4-dependent mechanisms augmented by C.pn infection promote the amount of atherosclerosis. Their role in plaque rupture remains to be studied. The method used involves ligation of CD40L on CD4+ T cells by CD40 on platelets; a similar mechanism within the plaque would be consistent with current data on pathogenesis of atherosclerosis.

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Further considerations on the mechanisms of NSAID hypersensitivity

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Previous studies on the participation of blood basophils to the syndrome of NSAID hypersensitivity, manifested by CD63 expression detected by flowcytometry and the release of sulfido leukotrienes (sLTs) have been reported before (CIA 2004). These results suggested that NSAID hypersensitivity is due to the joint effect of several factors, such as a local tissue inflammatory background raising cellular hypereactivity and expression of a pharmacogenetic abnormality.

We may now report the results of a European multicentric study involving 9 groups, over 130 validated NSAID-sensitive patients and 120 NSAID-tolerant controls. This study has confirmed the high sensitivity (over 70%) and specificity of the combined FLOW-CAST test. In addition, dose-response curves with various NSAIDs and simultaneous determination of various parameters, such as COX 1 inhibition, PGE2 synthesis , PGE2 receptors, LTC4 synthase, CysLT receptors and 15-HETE release demonstrate that the mechanism of basophil activation and release of various mediators may be an heterogeneous phenomenon.

Although the hypersensitivity reaction due to NSAIDs is based essentially on the release of sLTs, the fine pharmacological and enzymatic regulation of the NSAID-driven cellular activities is complex, individually programmed and possibly dependent upon several allelic genes and not a single one. This may be the reason why the fluctuating mechanism of acquired NSAID hypersensitivity has up to now escaped precise definition.

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Environmental prenatal factors of allergy in Lithuania

Ruta Dubakiene

The aim of the study was to evaluate the premises of allergic sensitization among pregnant women in Lithuania.

Methods. Total 205 pregnant women were interviewed with a questionnaire which included 235 questions about health status, housing conditions, socioeconomic status environmental factors, smoking habits, family history of atopy, allergies and other. Among them healthy persons were 121 (59,02%), allergic – 58 (28,29%), atopic – 26 (12,68%) (total 84 (40,88% in allergy / atopy group)

The cord blood and maternal milk from 20 healthy and from 20 atopic women was studied for specific IgE to 36 allergens panel and were determined using MAST CLA (Hitachi) technique.

To estimate the risk of various factors logistic regression was used and odds ratios with 95% confidence intervals were calculated, p values were taken into account too.

Results. Significant odds ratios are presented in a table.

		Allergic			Atopic		
Risk Factor	*Categories*	*Odds ratio*	*95% conf.interval*	*Pr>chisq*	*Odds ratio*	*95% conf.interval*	*Pr>chisq*
Allergies in family	No Yes	1 2,742	(1,431;5,251)	0,0022	1 5,757	(2,289;14,479)	0,0002
Father's allergy .	No Yes	1 4,261	(1,359,13,365)	0,0129	1 4,218	(1,049;16,962)	0,0426
Soft furniture carpets	No Yes	1 4,577	(1,02;20,52)	0,047	Not significant		
Pollinosis in family	No Yes	Not significant			1 7,429	(1,55;35,612)	0,0122

There other environmental factors in these groups, evaluated by logistic regression, were not significant such as: level of education; illness of brothers, sisters; living in a town or in a village; the age of building and it's nature; number of living persons in a flat; number of the rooms in a house; type of heating; air quality in a house (damp or dry); molds in a house; contact with animals and others. The differences between healthy women and allergic or atopic were significant in some cases. For example there was no significant difference between smoking in healthy and allergic/atopic women (p = 0.1209/0.8927), but great difference in family history of allergy (p< 0.0001). Specific IgE were found both in cord blood and both in maternal milk in atopic women with a significant difference between studied groups (p = 0.012 for cord blood, p< 0.003 for maternal milk). It was surprising, that the most frequent specific IgE among 36 was against mite Dermatophagoides pteronyssinus, which is the main environmental allergen in Lithuania. It was proved in our previous studies.

Conclusion. Environmental factors have a great influence on allergic sensitization during pregnancy.

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C3a and C4a: Complement Split Products Identify Patients with Acute Lyme disease

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Background: Lyme disease, caused in the USA by infection with the tick-borne spirochete *Borrelia burgdorferi*, is an increasingly prevalent infectious disease. Immune mediated inflammatory responses, both innate and acquired, are important in the eradication of the spirochete. Innate immune responses, especially complement, could serve as a marker for illness in patients seen shortly after a tick bite. No test is currently available to diagnose acute Lyme disease.

Methods: Factor B, C4, and C3 complement proteins were determined by nephelometry using specific anti-sera. C1q and C3d containing immune complexes were tested with ELISA kits provided by the Binding Site (C1q) and 1BL-Diagnostics (C3d). C2 protein was determined by diffusion in antibody impregnated agar gels. C3a des Arg was determined by kits provided by Quidel Labs, San Diego, CA and C4a des Arg by kits obtained from Pharmingen, B.D., San Jose, CA.

Patients: 31 consecutive acute Lyme disease patients, 14 with and 17 without an erythema chronicum migrans (ECM) skin rash seen by a physician within 48 hours of a tick bite, were matched with 20 consecutive tick bite patients without Lyme disease symptoms or ECM and 85 normal patients evaluated for routine physical exams.

In vitro testing: Pure cultures of *Borrelia burgdorferi* were added to normal human serum (NHS). Measurements of C3a, C4a and split products of Factor B were obtained at 60 minutes.

Results: Complement determinations C2, C4, C3, and Factor B were similar in all 3 groups. In acute Lyme disease, highly elevated levels of C3a and C4a were seen in all patients with ECM. In patients negative for ECM, 12 out of 17 patients had elevated C3a and 13 out of 15 patients had increased C4a. When *B. burgdorferi* was added to NHS, complement was activated by both the classical and alternative pathways.

Conclusions: C3a and C4a anaphylatoxins were significantly higher (p<.001) in the acute Lyme patients compared with the tick bite controls and normal patients. Measuring C3a and C4a should be helpful in differentiating acute Lyme disease from non-Lyme disease patients.

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Late onset anaphylactic reactions to Bacillus natto-fermented soybeans, which is well-known as Natto of traditional food in Japan

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Background: Fermented soybeans were recently reported to be a causative food of late-onset anaphylaxis. The hypothesized mechanism of late-onset anaphylaxis to fermented soybeans is due to delayed absorption or release into the bowel rather than an immunologic phenomenon.

Objectives: To clarify the clinical and laboratory features and to characterize the allergens in late-onset anaphylaxis to fermented soybeans.

Subjects & Methods: Six patients with suspected hypersensitivity to fermented soybeans from cliical history with late onset reactions to natto. In these patients, whom informed consent had been obtained, the skin prick-by-prick tests with fermented soybeans (natto) as is and provocation test by ingestion of natto were underwent. Additionally, specific IgE antibodies against fermented soybeans and the allergen proteins of fermented soybeans were detected by IgE-ELISA and by IgE-immunoblotting, respectively.

Results: Six patients were all man, aged from 27 to 42 years (mean age, 34.3 years). Six patients reported generalized urticaria and dyspnea, four, loss of consciousness, one, collapse, one, vomiting, and one diarrhea after ingestion of natto. The interval between ingestion of natto and onset of symptoms was 5 to 14 hour, mean 10 hour. All patients were positive in skin prick-by-prick test with natto as is. In one patient, provocation test by oral injestion of natto was positive with peak of histamine in blood 13 hours after its ingestion. In ELISA, all patients showed elevated levels IgE antibodies to the natto extracts. Furthermore, IgE-immunoblotting using 5 patients' sera showed six bands, of which three bands at 38, 28 and 26-kd were bound to sera from four patients.

Conclusion: Most cases with hypersensitivity after ingestion of natto would be IgE-mediated, late-onset anaphylactic reactions due to natto of fermented soybeans.

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Single nucleotide polymorphisms of CD14 is associated with the development of respiratory syncytial virus bronchiolitis in Japanese children

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Background: Respiratory syncytial virus (RSV) is the most important cause of lower respiratory tract disease in infant, and is also well known as the risk factor for the development of recurrent wheezing and bronchial asthma. Some defects of immune responses to RSV in infancy may well cause for severe RSV infection and also development of asthma. It was reported that the fusion protein of RSV binds to Toll-like receptor 4 (TLR4) and CD14. Therefore, genetic variations of these molecules may be associated with the difference in innate immune response to RSV and the development of post bronchiolitis airway diseases and/or airway allergy.

Objective: To explore genetic polymorphisms associated with the development of RSV bronchiolitis or recurrent wheezing after RSV bronchiolitis

Methods: We genotyped several SNPs of TLR4 and CD14 gene by PCR restriction fragment length polymorphism genotyping method, and investigated a relation between these SNPs and the development of RSV bronchiolitis or recurrent wheezing after RSV bronchiolitis in Japanese children.

Results: We did not find the Asp299Gly and Thr399Ile of the TLR4 gene in the Japanese population. We find that the distribution of genotype of CD14 C (-159) T in children with RSV bronchiolitis was the same with that in controls. In contrast, the distribution of genotype of CD14 C (-550) T in children with RSV bronchiolitis were significantly different from those in controls. Between infants with recurrent wheezing after RSV bronchiolitis and those without recurrent wheezing, there was no difference in the distribution of CD14 SNPs.

Conclusion: Genetic traits relating CD14 C (-550) T but not TLR4 might be important for the development of RSV bronchiolitis in the Japanese population.

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Predictive value of Phadiatop infant at 2 years for allergic sensitisation at 10 years of age.

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Background: Allergic sensitisation in early life is related to later allergic diseases. Phadiatop infant® (Pharmacia diagnostics, Uppsala, Sweden), a panel of specific IgE levels of prevalent allergens with relevance to early life atopic sensitization, has not previously been assessed for prediction of allergic sensitisation in later childhood.

Objectives: To assess predictive value of Phadiatop infant® in 2 years old children to allergic skin sensitisation at ten years.

Methods: In the Environment and Childhood Asthma birth cohort study, Phadiatop infant® was analysed by the Pharmaica CAP technique in 372 two years old children (59% with recurrent bronchial obstruction and 41 controls) who were re-examined at 10 years of age with skin prick test (SPT) to 15 common allergens (housedustmites, german cockroach, cat, dog, rabbit, birch, timothy grass, mugwort, cladosporium, alternaria, cows milk, egg, cod and peanut). The SPT was considered positive with the wheal of at least one allergen ≥3mm larger than the negative control.

Results: Positive and negative Phadiatop infant® (>0.35 PAU/L and 0-35 PAU/L) were found in 63 and 309 of the 2 year old children, respectively. At least one positive skin prick test (SPT) was found at 10 years in 271 (27.2%). children. The positive and negativ predictive value for Phadiatop infant® for at least one positive SPT at 10 years were 65.1 and 80.6, respectively, with an OR 7.73 (4.29-13.95) for pos SPT with positive Phadiatop infant® at two years.

	Phadiatop infant® positive n=63	Phadiatop infant® negative n=309	Concentration of Phadiatop infant®
SPT pos 10 y n(%)	41 (65.1)	60 (19.4)	2.90 (1.09-4.71) 95% c.i.
SPT neg 10y n(%)	22 (34.9%)	249 (80.6%)	0.17 (0.14-0.20) 95% c.i.
$p<0.001$ (chi^2)			

Conclusions: Phadiatop infant® at two years significantly predicts allergic sensitisation at 10 years, with a relatively high negative predictive value and a moderate positive predictive value.

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Hymenoptera venom allergy: Analysis of double positivity to honey bee and Vespula venom by estimation of specific IgE to species specific major allergens Api m1 and Ves v5.

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Background: In patients with Hymenoptera venom allergy diagnostic tests are often positive with venoms of the two hymenoptera species most often involved, honey bees (Apis mellifera) and wasps (Vespula spp). This causes problems in the selection of venoms for immunotherapy.

Methods: 100 patients each with a history of allergic reactions to either bee or Vespula stings within the last year and positive skin tests to the respective venom and 30 history and skin test negative controls were analysed for specific IgE to both venoms by UNICAP and ADVIA Centaur and to species specific recombinant major allergens Api m1, Ves v5 and Bromelain by ADVIA.

Results: Sensitivity for UNICAP was 1.0 for BV and 0.91 for VV, for ADVIA 0.98 for BV and 0,91 for VV. None of the 30 controls reacted to either BV or VV in both tests. Double positivity was observed in 62 of BV and 56 of VV allergic pts by UNICAP and 44 of BV and 20 of VV allergic pts by ADVIA. 56 pts were double positve only with UNICAP, 1 only with ADVIA.

None of the double positive pts with only one test had sIgE to the species specific allergen of the other venom.

Conclusions: Double positivity is frequently observed in hymenoptera venom allergic patients, signifcantly more often in UNICAP than ADVIA Centaur. sIgE to both species specific allergens Api m1 and Ves v5 indicates true double sensitization and thus immunotherapy with bee and Vespula venom.

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Tropomyosin in Invertebrates: Role in IgE Crossreactive Antibody Responses

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Tropomyosins are highly conserved proteins in invertebrates including mites, cockroach, shrimp and parasites. Tropomyosins from invertebrates are capable of inducing IgE antibody responses and have the potential to elicit cross-reactivity. Infection with *Ascaris lumbricoides* is an important risk factor for wheezing in children living in Brazil. We aimed to characterize tropomyosin from *A. lumbricoides* and to study IgE antibody responses to *A. lumbricoides* and cockroach using tropomyosin as a marker. A monoclonal antibody directed against *D. pteronyssinus* tropomyosin, which also recognizes cockroach and shrimp tropomyosins, showed strong binding to *A. lumbricoides* tissue on immunofluorescence. Using primers based on conserved sequences of cockroach tropomyosin in RT-PCR, we have amplified *A. lumbricoides* tropomyosin cDNA, which encodes a 240 amino acid protein with 90% to 96% identity to tropomyosins from other parasites, and 70% and 66% identity to mite and cockroach tropomyosins, respectively. Molecular modeling using the Modeller program revealed that tropomyosins from *A. lumbricoides* and cockroach share very similar tridimensional structure. Expression of recombinant protein was carried out in the *Pichia pastoris* system. Recombinant tropomyosins from *A. lumbricoides* and cockroach were used in chimeric ELISA to quantitate specific IgE antibodies in panels of sera from 132 children living in a parasite-endemic area, and 132 patients with asthma and/or rhinitis allergic to cockroach from our clinic. In children from a parasite-endemic area, IgE to tropomyosin was found in 101/132 (76.5 %) of sera; of those, 99 had IgE to both *Ascaris* and cockroach tropomyosin. Geometric mean (GM) levels of IgE to *Ascaris* and cockroach tropomyosin were 3.7IU/mL to both proteins. There was a significant correlation of levels of IgE to *A. lumbricoides* and cockroach tropomyosin ($r=0.65$, $p<0.0001$). Among cockroach allergic patients, presence of IgE to tropomyosin was found in 63/132 (47.7%), and of those 58 presented IgE to both *Ascaris* and cockroach tropomyosin, with GM levels of 7.5UI/mL and 4.9UI/mL, respectively. There was also a significant correlation of levels of IgE antibodies to both tropomyosins ($r=0.93$, $p<0.0001$). In conclusion, tropomyosins from invertebrates are important IgE-binding proteins. Recombinant tropomyosins could be used to investigate IgE antibody responses to *Ascaris* and cockroach.

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Low levels of plasma retinol in early infancy associate with subsequent development of atopic manifestations

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In newborn mammals, the gut immune response is assumed to play an important role in educating the maturing peripheral immune response through interaction with the gut commensal bacteria. T. Vitamin A has anti-inflammatory and immunomodulatory effects, and its deficiency results in impaired specific and innate immunity. Vitamin A is essential for inducing the gut-homing specificity on T cells. Since an impaired gut immune response in early infancy may contribute to the development of atopic sensitization, we looked for an association of plasma retinol concentrations and the subsequent development of allergic symptoms in healthy infants.

A cohort of 200 unselected, full-term newborns were followed-up from birth to age 20 years. The plasma retinol concentration was determined in the cord blood of 97 newborns, in 95 infants at ages of 2, 4 and 12 months, 155 children at age 5 years and 151 at age 11 years. The subjects were re-examined at ages of 5, 11 and 20 years, with assessment of the occurrence of allergic symptoms, skin prick testing, and measurement of serum total immunoglobulin E.

Subjects with allergic symptoms or a positive skin prick test in childhood or adolescence had lower retinol concentrations in infancy and childhood than symptom-free subjects. The difference was most pronounced at age 2 months. Retinol concentration at 2 months correlated inversely with positive skin prick test at ages of 5 and 20 years, and with allergic symptoms at age 20 years.

Thus, we assume that in case the newborn infant has a low concentration of retinol, the CD4-positive T cell homing to the gut is inhibited and the Th1 type responses initiated by the intestinal microflora and other antigens become weak. An environment favourable for the development of allergic sensitization may ensue. This may lead to the expression of the atopic phenotype and to the development of subsequent allergic symptoms.

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Inflammatory response in acute viral exacerbations of COPD

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Respiratory viruses are detected in more than 50% of all acute exacerbations of COPD. The inflammatory response in virus positive exacerbations has not been studied in detail yet. We investigated CRP, IL-6, IL-8, IL-10, IL-12p40, Interferon-^{3}, blood and sputum cell numbers in patients with acute exacerbation (n=86) and in stable disease (n=43) and correlated these parameters to virus detection in nasal lavage and/or induced sputum. 50/85 (59%) patients with acute exacerbation and 23/43 (54%) patients with stable disease were on systemic corticosteroids. The inflammatory response in patients without systemic corticosteroids was characterized by a systemic increase in CRP, IL-12p40 and the absolute number of blood leukocytes as well as a local increase in nasal IL-12p40, sputum IL-6 and IL-8 going along with an increase in sputum eosinophils during exacerbation. In patients with detection of respiratory viruses in nasal lavage local IL-6 production in sputum was significantly increased and negatively correlated to FEV_1. During acute exacerbation only Picornaviruses were found more often in patients without systemic corticosteroids (6/29 (20.7%) vs. 10/19 (52.6%), $p<0.025$). In conclusion this study firmly supports the view of increased local but also systemic inflammation in acute exacerbations of COPD. In virus associated exacerbations IL-6 plays a major role and may be a useful marker for the viral aetiology of exacerbation and possibly also a useful target for future therapeutic developments. Treatment with systemic corticosteroids seems to decrease susceptibility to Picornaviruses but does not affect susceptibility to other viruses.

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Tolerability of imipenem in patients with immediate hypersensitivity to penicillins

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Background: Imipenem is the prototype of the carbapenem class of β-lactam antibiotics, which is used primarily to treat serious polymicrobial infections or infections from highly resistant organisms in the hospital setting. Because of degradation in the kidney by the enzyme dehydropeptidase-I, imipenem is commonly used in combination with the enzyme inhibitor cilastatin. Administration of imipenem/cilastatin to patients with IgE-mediated hypersensitivity to penicillins who might benefit from this treatment is usually avoided because of a 47.4% (9 of 19 subjects) rate of cross-reactivity demonstrated in a single study on the basis of positive responses to skin tests with imipenem reagents.

Objective: To assess the cross-reactivity with imipenem/cilastatin in patients with documented IgE-mediated hypersensitivity to penicillins, as well as to evaluate the imipenem/cilastatin tolerability in patients displaying negative prophylactic skin tests.

Methods: We conducted a prospective study evaluating 112 consecutive subjects who had suffered a total of 143 immediate reactions (89 anaphylactic shocks and 54 urticarial and/or angioedematous manifestations) to penicillins, mostly aminopenicillins (amoxicillin, ampicillin, and bacampicillin). All patients were skin-test positive to at least one of the penicillin reagents tested: 48 were positive to both the classic penicillin reagents (penicilloyl-polylysine, minor determinant

mixture, and benzyl-penicillin) and semi-synthetic penicillins (ampicillin, amoxicillin, and piperacillin), 37 only to semi-synthetic penicillins, and 27 only to the classic penicillin reagents. Assays for specific IgE to penicilloyl G, penicilloyl V, ampicilloyl, and amoxicilloyl were positive in 45 (40.2%) of the 112 patients; 37 (82.2%) of these 45 patients had specific IgE to ampicilloyl and/or amoxicilloyl.

All subjects underwent skin testing with imipenem/cilastatin and negative subjects were challenged with it.

Results: One subject (0.9%) displayed a positive prick test to imipenem/cilastatin. Of the 111 subjects with negative skin tests to imipenem/cilastatin, one refused the challenge and 110 tolerated it. Challenges were not followed by full therapeutic courses.

Conclusions: Our data did not confirm the previously observed high rate of cross-reactivity between penicillins and imipenem/cilastatin. Therefore, the advisability of avoiding imipenem/cilastatin in patients with IgE-mediated hypersensitivity should be reconsidered. In those who especially require imipenem/cilastatin treatment, we recommend prophylactic skin tests, because negative results indicate tolerability.

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Kinetic study of the antigen-specific expression of lymphocyte activation markers CD69, CD25 and HLA-DR in patients with immediate reactions to Amoxicillin

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We assessed the kinetic of the antigen-specific activation of T cells in patients with immediate reactions to amoxicillin, by the determination of the early activation marker CD69 and the late markers CD25 (IL2r) and HLA-DR.

Thirty-six patients allergic to amoxicillin with IgE-mediated reaction, anaphylaxis and/or urticaria-angioedema within half an hour after administration of amoxicillin, and positive skin tests, and 16 healthy controls who tolerated betalactams were studied.

After *in vitro* incubation of the mononuclear cells isolated from peripheral blood with amoxicillin at different times 0, 4, 24 and 48 hours, the cells were labelled with the following mononuclear antibodies to be assessed subsequently by flowcytometry in a FACScan (Becton Dickinson):

For the determination of CD69, CD25 and HLA DR expression in lymphocytes T CD4+, the following triple labelings were used respectively:

CD3 FITC /CD69 PE and CD4 Percp Cy5.5.

CD3 FITC /CD25 PE and CD4 Percp Cy5.5.

CD3 FITC / HLA-DR PE and CD4 Percp Cy5.5.

The results are expressed as % cells expressing CD69, CD25 or HL-DR after amoxicillin stimulation (0.625 mg/ml).

In the group of patients the CD69 and HLA-DR expression increased significantly at 4 hours of culture with amoxicillin. At 24 hours of culture we found a significant increase of CD25 (IL2r) and HLA-DR. In the control group only a slight increase of CD25 expression has been observed at 24 hours.

Patients (n=36)

	Time 0h	Time 4h		Time 24h			Time 48h			
	SI (mean±SD)	SI (mean±SD)	p*	SI (mean±SD)	p*	p**	SI (mean±SD)	p*	p**	p***
CD 69	0.14±0.36	13.08±30.44	**0.001**							
CD 25	2.13±2.41	1.12±0.62	ns	8.04±27.90	ns	**0.018**	1.70±1.58	ns	**0.007**	ns
HLA-DR	1.61±1.8	1.11±0.9	**0.001**	5.08±14.74	**<0.001**	**<0.001**	2.12±3.72	ns	**<0.001**	ns

Controls (n=16)

	Time 0h	Time 4h		Time 24h			Time 48h			
	SI (mean±SD)	SI (mean±SD)	p*	SI (mean±SD)	p*	p**	SI (mean±SD)	p*	p**	p***
CD 69	0.19±0.32	0.55±0.55	ns							
CD 25	0.69±0.51	1.40±1.72	ns	1.96±1.40	**<0.05**	ns	2.65±3.80	ns	ns	ns
HLA-DR	2.84±1.32	0.88±1.29	ns	1.75±3.91	ns	ns	4.65±12.82	ns	ns	ns

p* with respect to time 0 hours

p** with respect to time 4hours

p*** with respect to time 24 hours

To conclude, the patients with immediate reactions to amoxicillin have an early activation of T CD4+ cells that significantly increase the expression of CD69 at 4 hours. Also we found a late activation with the increase of expression CD25 and HLA-DR at 24 hours.

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Autoreactivity in atopic dermatitis due to molecular mimicry with fungal allergens

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Background: The opportunistic yeast *Malassezia* belongs to the normal cutaneous flora but can also cause IgE-mediated sensitization in patients suffering from atopic eczema.

Objective: To investigate sensitization to *M. sympodialis* in a large allergic population and, by the use of recombinant *M. sympodialis* allergens, to characterize particular allergens involved in both, extrinsic and intrinsic (nonatopic) atopic eczema.

Methods: Totally 655 individuals with different allergic diseases and 38 healthy controls were investigated by ImmunoCAPm70 and skin prick tests with a crude *M. sympodialis* extract. In the atopic eczema patients we further performed skin prick tests, atopy patch tests, ELISA and a PBMC proliferation assay with the six recombinant *M. sympodialis* allergens rMala s 1 and rMala s 5 - 9.

Results: In 52 of 97 patients with atopic eczema (54%) specific serum IgE against *M. sympodialis* was detectable. Almost no reactivity to *M. sympodialis* was seen in patients suffering from other allergic diseases (4/558) and no reactivity at all was seen in the healthy control group (0/38). Skin tests with the recombinant allergens of *M. sympodialis* showed variable recognition patterns against the different molecular structures with a predominant sensitisation to rMala s 1, 5, 6 and 9, confirmed also by specific serum IgE to these allergens. Interestingly, IgE- and T-cell mediated reactivity against *M. sympodialis* was also found in patients with the intrinsic form of atopic eczema.

Conclusion: Sensitization to *M. sympodialis* is specific for atopic eczema patients and occurs in both the extrinsic and intrinsic (nonatopic) variant of eczema, what may have important diagnostic and therapeutic consequences. Recombinant yeast allergens represent a useful tool to study the involvement of defined molecular structures and differential sensitization patterns in the pathogenesis of atopic eczema.

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Comparison of Microarray-based IgE Profiling with Established Diagnostic Methods in Patients with Latex Allergy.

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Rationale: Immediate reactions to natural rubber latex (NRL) still have to be considered a clinically relevant phenomenon with up to 17% of exposed health care workers. Routine in vitro and in vivo tests for latex allergy employ non-standardized allergen extracts. For that reason sensitivity and specifity are subjects to fluctuation. However, several latex proteins have been characterized and synthesized as recombinant allergens and are available for a component-resolved diagnosis in patients with latex allergy.

Methods: We studied 63 adult patients suffering from clinically significant symptoms as defined by latex allergy and in whom type I sensitization to NRL had priorily been confirmed by an established fluorescence enzyme immunoassay using allergen extracts. We applied a new microarray-based method in which a computer-assisted qualitative and quantitative analysis of interacting human IgE antibodies with an array of recombinant allergens was performed incubating only 20 µl of patient serum on a solid-phase chip. In addition we detected specific IgE against latex with an established fluorescence immunoassay (UniCAP, Pharmacia) by using recombinant latex allergens, too, and correlated the results of the two methods.

Results: The spearman correlation coefficient showed a moderate degree of correlation between chip-analysis and the established enzyme immunoassay in the case of rHev b 5 and 6 (0,64 and 0,73). Other latex-components showed weak correlations.

Conclusion: Microarray-based method shows moderate correlation with established diagnostic tools by using recombinant allergens. Additionally, this technique may be used to improve the uncertain diagnostic of latex allergy by using minimal amounts of serum.

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Epidermal caspase-3 cleavage associated with interferon-γ expressing lymphocytes in acute atopic dermatitis lesions

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Background: Keratinocyte apoptosis mediated by Fas/Fas ligand molecular interactions and subsequent caspase activation is believed to play an important role in the pathogenesis of atopic dermatitis (AD), in particular for the formation of spongiosis. To estimate epidermal caspase activation in normal and AD skin under in vivo conditions, we analyzed caspase-3 cleavage by immunohistology.

Methods: Immunofluorescence staining was done using an antibody against the large fragment of the cleaved form of caspase-3 as well as antibodies against IFN-γ, CD4 and CD8 and analyzed by confocal microscopy.

Results: In normal skin as well as non-lesional AD skin, we detected caspase-3 cleavage in single cells of the basal layer. In contrast, in acute lesional AD skin, we obtained not only evidence for increased expression of cleaved caspase-3 in keratinocytes of the basal layer, we also observed caspase-3 cleavage in one or more layers of the spinous cell layer, in particular in spongiotic areas. Short-term topical treatment of the skin lesions with tacrolimus or pimecrolimus abolished the expression of cleaved caspase-3 in the spinous layer. Moreover, epidermal caspase-3 cleavage correlated with the numbers of dermal interferon (IFN)-γ expressing CD4+ and CD8+ lymphocytes in skin lesions of AD patients, supporting the view that IFN-γ is important for the activation of proapoptotic pathways in keratinocytes.

Conclusion: These data suggest that caspase-3 cleavage in the spinous layer of the epidermis is a pathologic event contributing to spongiosis formation in AD, whereas the presence of cleaved caspase-3 in basal cells is physiological and may mediate apoptosis by which cell numbers within the process of epidermal renewal are controlled.

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Eosinophilic Esophagitis: Escalating Epidemiology?

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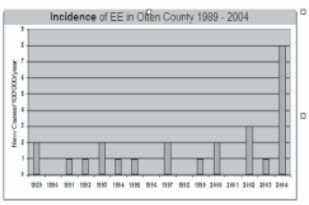
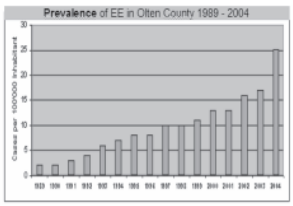
***Background*:** Eosinophilic esophagitis (EE), a chronic inflammatory disorder of the esophagus, was originally considered rare, but is now increasingly recognized. The leading symptom in adults is dysphagia for solids with the imminent risk of food impaction. The diagnostic criterion is a dense eosinophilic infiltration of the esophageal epithelium. The inflammation may induce esophageal tissue damage and subsequent fibrosis with ensuing narrowing and stricture. Some demographic data exist for pediatric patients, but not for adults, the primary target population of this study.

***Methods*:** We report on 25 adult cases selected from a continuing database commenced in 1989 that prospectively enrolls EE patients. All patients presented here lived in Olten County, Switzerland, an area having only one gastroenterology and pathology center, approximately 100,000 inhabitants, and having undergone no relevant demographic changes within recent decades. Database inclusion criteria are 1.) Typical history; 2.) Consistent endoscopic abnormalities; and 3.) Peak infiltration of the esophageal epithelium with eH24 eosinophils/hpf. GERD is excluded clinically and endoscopically.

***Results*:** Throughout an almost 16-year observation period, an average annual incidence of 1.438/100,000 inhabitants was noted (range 0-6) with a marked increase in newly-diagnosed cases during the last years (Figure A), leading to a current prevalence of 25/100,000 inhabitants (Figure B).





Given the stability of demographic and recording conditions, it is very likely that this remarkable trend reflects a real increase in EE and not just enhanced awareness. Furthermore, our frequency calculations should be considered a minimum as probably only the "tip of the iceberg" has been recognized; many oligo- or asymptomatic cases may remain undiagnosed.

***Conclusions*:** Our data indicate that, in adults, EE may be one of the leading causes of dysphagia and, once believed to be a rare anomaly, occurs at a considerable frequency and may soon reach that of chronic inflammatory bowel diseases.

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A geriatric murine model of food allergy

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Background and Aims: Despite the steadily increasing number of elderly, little attention is given this age group in allergological studies. Recently, animal studies have indicated a defect in type 2 cytokine responses and in IgE isotype switching in senescence. However, protein feedings under hypoacidic conditions efficiently induce antigen-specific IgE and food allergy in BALB/c mice. Therefore, in the present study we aimed to apply this effective immunization regimen to investigate whether at all the development Th2 type immune response can be achieved in immunosenescent BALB/c mice.

Methods: Extracted celery proteins, in a first step evaluated for digestion resistance in simulated gastric fluid experiments, were used to feed senescent mice (age 18 months) with or without concomitant acid-reduction treatment (proton pump inhibitor in combination with Sucralfate). Serum samples of immunized mice were screened for celery-specific antibodies, which were further assessed for their functionality in RBL assays. Further, mice were subjected *in vivo* type I skin tests.

Results: For most celery proteins (except a 60 kDa protein) the stomach acts as a physiological gate-keeper, as they were degraded within a few seconds by simulated gastric fluid. Therefore, pharmacological acid reduction should hinder pepsin activation and, thereby, affect the consecutive immune responses towards celery. When orally immunizing the aged mice with the celery proteins we could induce high titers of antigen-specific IgG1 in both mouse groups, independent on their gastric digestion capability. However, IgG2a could be detected only in the serum of animals being fed with celery under normal gastric conditions, but not when gastric digestion was inhibited through medication. Importantly, celery-specific IgE was induced only in the acid-suppressed animals. These IgE antibodies were functionally relevant, as evaluated by positive RBL assays and positive type I skin tests.

Conclusion: Our data indicate that an antigen-specific Th2 response, including a functional IgE switch and positive skin test reactivity, can effectively be induced in geriatric mice when dietary proteins remain undigested.

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Sensitisation to different pollens and allergic disease in 4 year old Swedish children

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Background Although the relationship between sensitisation to different inhalant allergens in adolescents and adults has been intensively studied, information concerning sensitisation in children is scarce in particular to pollens.

Investigation In 4-year-old children to elucidate the pollen IgE antibody profile (birch only, timothy only and combinations of 3 pollens (birch, timothy or mugwort) and to relate the results to other inhalant and food allergens, as well as the presence of allergic diseases.

Methods A total of 2,551 four-year-old children belonging to a prospective birth cohort, which has been followed longitudinally (BAMSE), were investigated with respect to IgE antibodies to pollen and other inhalant and food allergens, and expression of allergic disease, based on questionnaire data.

Results Eleven percent (n=285) of the children were sensitised to pollen. Birch was the dominating cause of pollen sensitisation (birch sensitisation only, n=133); followed by timothy grass pollen (n=56) and a combination of two (n=64) or three (n=30) pollens. Two children had IgE antibodies to mugwort alone. Children sensitised only to birch demonstrated nearly a three times higher frequency of IgE reactivity to cat, horse and mould ($p<0.05$) and a doubling to dog ($p<0.05$) compared to those sensitised only to timothy. The highest frequencies of IgE reactivity to food were found in the group of children sensitised to the combination of birch, timothy and mugwort pollen. Children sensitised to two or three pollens reported the highest frequency of asthma and/or rhinitis and/or eczema. A significantly higher frequency of symptoms of allergic disease was found among children sensitised to birch only or to any combination including birch compared to children sensitised to timothy only.

Conclusion Our results demonstrate that birch is the dominating source of pollen sensitisation at the age of four in Sweden. This might associate with the pattern of sensitisation to other inhalant and food allergens as well as influence on the expression of allergic disease in this particular age group.

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More than 50% of positive challenges with foods are associated with late eczematous reactions in atopic dermatitis

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It is well-known that foods such as cow's milk and hen's eggs often cause immediate reactions in sensitized infants with AD, whereas pollen-related foods are of greater importance in older patients. The relevance of food allergy for the course of eczema is poorly defined. In this study we evaluated the outcome of oral food challenges in different age groups and focussed on late eczematous skin reactions after 6 to 24 hours. We compared the clinical outcome of the provocation tests to in vitro parameters (specific IgE, T-cell activation) and performed T-cell cloning experiments with cells from the blood and from lesional skin in selected patients.

First we analyzed 268 double-blind placebo-controlled food challenges (DBPCFC) to cow's milk, hen's egg, wheat and soy in 151 children with AD (median age 2 years). 37% of the challenges were related to a clinical reaction. Isolated late eczematous reactions were observed in 15% of positive challenges. 37% of the positive challenges were associated with late eczematous responses which followed immediate-type reactions. The parents´ histories of food-induced eczema and specific IgE were often false positive.

In a second series we analyzed the outcome of DBPCFC with birch pollen associated foods (apple, carrot, celery and/or hazelnut). These DBPCFC had been performed on 146 adolescents (>12 years) or adults with AD sensitized to birch pollen allergens. 33% of the patients showed a clinical reaction. Isolated eczematous reactions were seen in 20% of positive challenges. 44% of the positive challenges were associated with late eczematous responses which followed immediate-type reactions. Of note, the majority patients was not aware of a birch pollen related food allergy prior to DBPCFC. A possible role of allergen-specific T-cells in eczema was confirmed by the detection of food-reactive T-cells in the circulation and in lesional skin.

In conclusion, more than 50% of positive reactions to foods are associated with late eczematous reactions. Due to the poor reliability of the patients´ histories and food-specific IgE tests alternative diagnostic tools for the detection of food induced eczema have to be evaluated.

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Delineation of IL-13 effects in skin inflammation of atopic dermatitis

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Skin inflammation in atopic dermatitis (AD) is characterized by the predominant infiltration of Th2-cells in lesional skin. Recent studies have described that IL-13 acts on epithelial cells via inducing chemokines and thus enriching inflammatory cells to the site of inflammation. However, the mechanism of recruitment of these cells in lesional skin of AD is not yet fully elucidated. In this study we investigated the role of IL-13 stimulated human primary keratinocytes (HPKs) in recruitment of lymphocytes and further delineated the mechanism of enrichment of these cells. In migration assays, we observed preferential enrichment of $CCR4^+CD4^+$ T-cells towards IL-13 stimulated HPKs. Interestingly, $CCR4^+CD4^+$ T-cells from AD showed a higher chemotactic response than those from healthy individuals. We observed a marked and significant increase in the expression of CCL-22/MDC but not CCL-17/TARC and CCL5/RANTES in IL-13 stimulated HPKs as compared to unstimulated cells. Blocking of CCL-22/MDC in IL-13 stimulated HPKs by a neutralizing antibody resulted in 70-90% inhibition in migration of CCR4+CD4+ T-cells. Moreover, IL-13 upregulated IFNγ induced chemokines such as CCL-2/MCP-1 and CCL-5/RANTES in HPKs. Moreover, IL-13 stimulation of HPKs induced selective expression of functional matrix metalloproteinase-9 (MMP-9) as detected by an bioactivity assay. In addition, IL-13 downregulated the expression of E-cadherin on HPKs. Finally, we have demonstrated a concomitant expression profile of MMP-9 and IL-13 in biopsies of lesional allergic eczematous skin. Taken together our data suggest that IL-13 stimulated HPKs participate in a positive feedback loop by preferentially enriching Th2-cells in lesional skin of acute AD patients. In the chronic phase, IL-13 may act in synergy with IFNγ resulting in lymphocytes recruitment of a mixed phenotype at the site of inflammation, thus contributing to the chronification of eczema. In addition, IL-13 induced epidermal tissue remodeling (MMP-9 production and E-cadherin downregulation) may play a role in skin inflammation by means of facilitating emigration of LCs into the lymph node and infiltration of inflammatory cells into the epidermis.

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Ten years experience with epidermal dendritic cell phenotyping as a diagnostic tool

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The differential diagnosis of atopic dermatitis (AD) is largely based on clinical features. Skin prick and patch tests do not assess single lesions, whereas histological analysis shows limited diagnostic power. Therefore we proposed epidermal dendritic cell phenotyping (EDCP) as a diagnostic tool. This method is based on multi parameter flow cytometric analysis of myeloid and plasmacytoid dendritic cells (DC) in epidermal cell suspensions prepared from lesional skin biopsies. The underlying concept is that the immunophenotype and distribution of the dendritic cell subsets reflect the disease specific microenvironment of the clinically defined skin diseases and that EDCP may help in differential diagnosis of inflammatory skin diseases on the level of single lesions.

During the last 10 years, we have prepared epidermal singe cell suspensions from 952 skin biopsies of extrinsic and intrinsic atopic dermatitis, psoriasis, contact dermatitis, eczema herpeticum and numerous other skin diseases for EDCP. The expression of Fc-receptors, adhesion molecules and MHC complexes were quantitatively determined, correlated with the clinical and histological data from the patient's charts and analyzed statistically to re-evaluate and improve our initially proposed diagnostic criteria.

Myeloid DC accumulated in skin lesions of intrinsic AD and lichen planus, whereas lupus erythematodes showed highest plasmacytoid DC numbers. Extrinsic AD was identified by an expression ratio of FceRI/CD32 on myeloid DC exceeding 1.5 with a sensitivity of 75% and a specificity of 91%. Psoriasis was identified by an expression ratio of CD64/CD11b on IDEC exceeding 0.3 with a sensitivity of 81% and a specificity of 89%. Cases of Netherton syndrome and persistent light reaction fulfil the EDCP criteria for AD, whereas ichthyosis congenita, eosinophilic cellulitis, chronic hand dermatitis and Dorfman-Chanarin-syndrome do not.

We conclude that immunophenotyping of epidermal dendritic cells may be a helpful procedure for differential diagnosis of inflammatory skin diseases, as well as an experimental procedure for the investigation of skin immunobiology.

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Detailed sequence and haplotype analysis of the beta-2 adrenergic receptor gene in Caucasians and African Americans

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Rationale: Since β_2 adrenergic receptor (β_2AR) variations appear to alter an asthma patient's response to beta-agonist therapy, it is important to fully characterize β_2AR gene variations and haplotypes in different ethnic groups. Clinical studies show that individuals

homozygous for Arg^{16} have an unsatisfactory beta-agonist response versus individuals homozygous for Gly^{16}.

Methods: A region -3470 bp 5' of the ATG start site to +1886 bp in the 3' UTR of the β_2AR gene was re-sequenced in 429 Caucasians and 240 African Americans asthma cases and controls.

Results: Forty-nine polymorphisms were identified, twenty-one of which are novel. Haplotypes were constructed using polymorphisms with frequencies ≥0.04 and LD measured. LD was strong across the β_2AR gene, except for the 3'UTR region. Seven Caucasian haplotypes containing Arg^{16} were differentiated exclusively by 3' UTR variations. Ten African American haplotypes containing Arg^{16} were differentiated by 3' UTR and promoter variations. Three novel promoter insertion/deletions were found in African Americans. The $Thr^{164}Ile$ variant was found exclusively in Caucasians while the $Ser^{220}Cys$ variant was found exclusively in African Americans. A potentially important 3'UTR polymorphism consists of an interrupted poly(C) repeat varying in size and frequency. This poly(C) region lies adjacent to an AU rich element, which is known to affect β_2AR mRNA stability and translation in animal models. It is not clear what affect the hyper-variable 3'UTR region may have on regulation of human β_2AR expression. Significant haplotype associations for % predicted FEV_1, % predicted FVC, and $(FEV_1/FVC)^2$ were found in African Americans

Conclusion: These new observations provide opportunity to perform detailed comparison to beta-agonist responses and genotype and haplotype differences in the β_2AR gene.

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Gene-by-gene interactive for asthma: Use of family studies to identify linked regions

Deborah Meyers

As with many common diseases with a genetic component, genome wide linkage analyses have been performed in multiple sets of families but often with conflicting results. Gene-by-gene interactive effects may contribute to the lack of reproducibility in genome-wide linkage scans. A genome-wide linkage screen for asthma susceptibility genes has been performed on the families in the NHLBI-funded Collaborative Study of the Genetics of Asthma (CSGA) (Xu 2001). The 144 Caucasian families and 107 African-American families were ascertained through two siblings with asthma and all family members were characterized and genotyped. We have now performed a genome-by-genome linkage analyses on these same families using ordered subset analysis to determine the level of significance. The highest lod (lod difference between the conditional and unconditional lod scores) in the Caucasian families was for chromosomes 2 and 14 (lod = 4.21, p = 0.0005) and for chromosomes 15 and 5 in the African-American families (lod = 3.79, p = 0.0001). The second highest lod both in the African-American and Caucasian families was for chromosome 20p with chromosome 6 in the African-American families (lod =3.63, p<0.0005) and with chromosome 8 in the Caucasian families (lod = 4.19, p=0.0002). The linkage to 20p is in the region where the ADAM33 gene has been cloned for asthma (VanEerdewegh 2002) and replicated in a CSGA case-control population (Howard 2003). In summary, there was significant evidence for interaction between chromosomal regions that have been previously reported in genome wide screens although not observed previously in the CSGA population. These results suggest that interactive effects from previously identified chromosomal regions may contribute to asthma susceptibility. In addition, there was evidence for novel regions. This approach should be very useful in defining a more homogenous set of families for fine mapping and gene identification.

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Maternal smoking in pregnancy is associated with suppression of neonatal TLR-mediated microbial responses and this effect is increased by maternal allergy.

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Background: Early life exposures have critical effects on the developing immune system and can increase allergic predisposition. In particular, there is mounting interest in factors that influence developing responses to microbial agents. This study addressed the effects of two major maternal factors on early Toll-like receptor (TLR)-mediated microbial responses, namely maternal allergy and smoking in pregnancy. As well as first-line microbial defence, innate TLR-mediated pathways modulate subsequent specific immune response and are essential for normal immune maturation.

Methods: In a prospective birth cohort (n=122), we compared cord-blood immune responses of neonates of smoking (n=60) and non-smoking (n=62) mothers. These groups included equal numbers of allergic women (50% and 49% respectively). Neonatal cytokine responses were assessed to optimal doses of TLR2 ligand (Pansorbin 0.1%), TLR3 ligand (Poly [I:C] 30ug/ml), TLR4 ligand (lipopolysaccaride [LPS] 10ng/ml) and TLR9 ligands (CpGB and CpGC 1.66 ug/ml), as well as house-dust-mite ([HDM] 20ug/ml), betalactoglobulin ([BLG] 100ug/ml) and purified-protein-derivative (PPD 10ug/ml).

Results: Infants of smoker showed significantly attenuated TLR-mediated responses compared to infants of non-smokers, including lower responses following TLR2 (TNFα p=0.004; IL-6 p=0.045; IL-10 p=0.014), TLR3 (TNFα p=0.044) TLR4 (TNFα p=0.034) and TLR9 (IL-6 p=0.046) activation. These infants also had significantly lower IL-6 responses to antigen-specific stimulation with PPD (p=0.002), HDM (p=0.026), and BLG (p=0.047) compared with infants of non-smokers. Maternal allergy did not have significant independent effects on TLR responses, although there was a trend for lower TLR2 (IL-6 and IL-10), TLR4 responses (IL-10) and PPD (IL-6) responses in neonates of atopic mothers. However, the inhibitory effects of smoking on TLR responses were significantly greater if mothers were atopic. This potentiating effect of maternal allergy was most apparent for TLR2 (IL-6), TLR3 (TNFα) and TLR4 (TNFα) responses.

Conclusions: This demonstrates that in addition to effects on developing airways, maternal smoking in pregnancy also has significant immunologic effects that could contribute to increased risk of respiratory infections and asthma. These effects appear to be mediated through effects on TLR-mediated innate response pathways that also promote regulatory pathways in the inhibition of allergic immune responses. This highlights that other environmental interactions are highly relevant to the "hygiene hypothesis".

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Th2 Micromilieu Generated by Pollen Associated Lipid Mediators (PALMs)

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We recently demonstrated that pollen liberate bioactive lipid mediators with chemical and functional similarities to leukotrienes and prostaglandins – the pollen associated lipid mediators (PALMs). The prostaglandin-like mediators were characterised as dinor isoprostanes, the phytoprostanes, derived from linolenic acid. We demonstrated that PALMs block the LPS/CD40L induced IL-12 production in human dendritic cells leading to a Th2 pattern in the ensuing T-cell response. Herein we investigated the effects of water soluble factors from birch and grass pollen grains on chemokine release from keratinocytes and dendritic cells. Human primary keratinocytes from non–allergic patients were exposed to aqueous pollen extracts (APE). In an *in vitro* inflammatory skin model primary human keratinocytes were prestimulated with TNF-± (50 ng/ml) and subsequently incubated with aqueous pollen extracts (APE). Effects on chemokine and cytokine production was investigated by real-time PCR and ELISA. APE significantly induced the Th2 chemokines CCL17 and CCL22 in resting keratinocytes while Th1 chemokines such as CXCL10 and CXCL11 were not expressed after APE stimulation. Furthermore, APE blocked the TNF ± induced production of CXCL10 and CXCL11 while the TNF-± induced expression of CCL22, CCL5 and CCL17 was further enhanced by APE. Similar results were achieved in human dendritic cells. Here the LPS-induced release of Th1 chemokines (CXCL10, CCL5) was reduced while the Th2-chemokines (CCL17 and CCL22) were enhanced by APE. Protein and mRNA data were confirmed by functional studies showing that APE blocked the migration of Th1 cells and favoured the chemotaxis of Th2 cells towards dendritic cells (compared to LPS-stimulated DCs). Since LPS and TNF-α are primarily involved in NF-kappa b signalling these results prompted us to investigate the effect of APE on the NF-kappa b signal cascade. Preliminary results show that APE block the NF-kappa b signalling in human dendritic cells. In summary, our results demonstrate that pollen associated lipid mediators (PALMs) act as important regulatory mediators which generate a Th2 promoting micromilieu leading to generation and infiltration of Th2 cells into the skin of predisposed individuals.

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Co-stimulation of mast cells via FcεRI and Toll-like Receptors Markedly Augments Production of Inflammatory Cytokines

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Mast cells mediate both IgE-dependent allergic reactions and protective responses to acute infections possibly through the activation of Toll-like receptors (TLRs). Clinical reports indicate that allergies are exacerbated by viral/bacterial infections. We investigated whether or not TLR ligands act in synergy with FcμRI receptor to enhance release of inflammatory mediators from mast cells.

METHODS: Release of inflammatory mediators was studied by measurement of degranulation, release of arachidonic acid, and production of cytokines in cultured MC/9 and primary mouse bone marrow-derived cells. Signaling events were assessed by measurement of kinase activities, immunoprecipitation, immunoblotting, and binding of transcription factors to oligonucleotide arrays.

RESULTS: We find that antigen interacts synergistically with TLR2/TLR1, TLR2/TLR6, and TLR4 ligands to markedly enhance production of inflammatory cytokines namely, TNFα, Interleukin (IL)-6, IL-12, and IL-13. With low concentrations of antigen and TLR ligands up to 50-fold increase in cytokine production was observed. However, the TLR ligands neither stimulated degranulation and release of arachidonic acid nor influenced such responses to antigen probably because these ligands failed to generate a necessary calcium signal. The enhanced cytokine production could be attributed to synergistic activation of mitogen activated protein kinases in addition to the engagement of a broader and more effective repertoire of transcription factors for cytokine gene transcription. Of note, clinically relevant concentrations of the glucocorticoid, dexamethasone, effectively suppressed responses to both antigen and TLR ligands through induction of inhibitory regulators of signaling.

CONCLUSIONS: The synergistic interactions of TLR ligands and antigen might have relevance to the exacerbation of IgE-mediated allergic diseases by infectious agents and to the treatment of these diseases.

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Allergo-Oncology: the role of IgE in tumor defense

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Background and Aims: Allergology and Oncology are rarely connected, but some epidemiological studies indicate an inverse correlation between the incidences of allergy and tumor disease. Moreover, one out of 200 asthmatic patients treated with the anti-IgE antibody omalizumab developed malignancies including breast, skin and prostate cancer during the median observation period of the study of one year. A basic function of IgE antibodies may thus be to control tumor growth. Consequently, we aimed to analyse *in vitro* whether IgE directed against tumor associated antigens can execute tumor cell killing.

Methods: Trastuzumab is a monoclonal antibody directed against the tumor antigen HER-2 (Human Epidermal growth factor Receptor-2), and is presently used for passive immunotherapy of breast cancer. We have previously generated mimotopes (epitope mimics) for trastuzumab and applied them for intraperitoneal immunizations and induction of IgG antibodies with trastuzumab-like properties. Using these trastuzumab mimotopes we immunized BALB/c mice via the oral route under simultaneous reduction of gastric acid by a proton-pump inhibitior and sucralfate. As demonstrated in preceding food allergy studies this feeding regimen effectively induces Th2 immune responses.

Results: As expected, oral immunizations under hypoacidic conditions resulted in the formation of IgE antibodies towards HER-2 monitored in ELISA. This IgE effectively sensitized RBL cells expressing FcεRI but not Fcγ- receptors. Triggering could be performed with the HER-2 overexpressing breast cancer cells SKBR-3, but not with a control cell line. Further, the supernatants of triggered RBL cells acted cytotoxic on SKBR-3 cells as determined by a non-radioactive cytotoxicity assay. Thus, we demonstrate here for the first time that IgE antibodies mediate tumor cell killing via FcεRI-positive effector cells.

Conclusions: An oral mimotope vaccine enables directed and epitope-specific induction of IgE against tumor antigens. This novel type of tumor vaccine exploits IgE as an effector molecule of outstanding specificity, affinity, and utilizing a panel of distinguished executing cells. Based on our data, we suggest that also the natural function of IgE may be the control of tumor growth.

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Conformational change in the IgE-FcεRI interaction as a target for inhibitor design

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The interaction between IgE and its high-affinity receptor FcεRI is a target for the design of inhibitors of the allergic response, as demonstrated by the efficacy of the anti-IgE antibody Omalizumab. Our X-ray structure determination of the Fc region of IgE revealed, unexpectedly, an asymmetrically and acutely bent conformation, with an extensive interface between Cε2 and Cε3 domains, and even contact between Cε2 and Cε4 domains. The crystal structure of the complex between a Cε3-Cε4 fragment and the soluble receptor (Fcε3-4/sFcεRIα) had earlier revealed that both Cε3 domains engaged with receptor in the high-affinity complex, but in the bent IgE structure, only one of the two Cε3 domains is accessible for interaction with receptor. This implies that a substantial conformational change in both the Cε2 and Cε3 domains of IgE must occur upon receptor engagement, and our NMR analysis of the Cε2:sFcεRIα interaction supports this hypothesis. "Allosteric inhibition", rather than direct blocking of the interaction, is thus an alternative and promising strategy. In order to determine the effect of inhibiting this conformational change upon receptor binding, and its physiological consequences, we generated a version of IgE Fc lacking the inter-heavy-chain disulphide bridge. This modified Fcε3-4, which engages sFcεRIα through only a single Cε3 domain, displayed an affinity 1000-fold lower than the native affinity; this is therefore the potential effect of an inhibitor of the conformational change. We have also shown that a single point mutation in the Cε3 domain of IgE, Arg334Ser in the receptor-binding site, reduces the affinity of IgE for FcεRI 50-fold. In a transgenic mouse model of passive cutaneous anaphylaxis, in which the mouse expresses human FcεRIα and is capable of binding only human IgE, sensitization with the mutant Arg334Ser IgE caused a highly significant reduction in passive cutaneous anaphylaxis compared to wild-type IgE. Thus inhibition of the conformational change can be expected to have significant efficacy *in vivo*. The production of fluorescently labeled IgE-based reagents to screen for small molecule inhibitors of the conformational change is now underway.

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IgE switching and synthesis is regulated locally in the bronchial mucosa in atopic and non-atopic asthma

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BACKGROUND: Asthma is a chronic inflammatory disease of the airways that is characterized by IgE-dependent mast cell activation and tissue eosinophilia. The location of IgE switching and IgE synthesis in the target organ in asthma remains an unresolved question. This question is important because IgE antibodies mediate the allergic response in asthma. We have investigated this problem in patients with atopic and non-atopic asthma compared with non-asthmatic controls.

METHODS: Our aim is to address IgE regulation with 6-8 bronchial biopsies from each of the 10 patients representing 4 groups: atopic asthmatics (AA), non-atopic asthmatics (NA), atopic non-asthmatic controls (AC) and non-atopic non-asthmatic controls (NC). PCR-based methodology is used to analyze different molecular markers closely associated with ongoing class switch recombination (CSR), germline transcripts (GLT), switch circle transcripts (CT), activation-induced cytidine deaminase (AID) and mature IgE mRNA expression. The study is performed with the approval of the Guy's Hospital/King's College Ethics Committee and the patients' written informed consent.

RESULTS: ε GLT expression was observed in 7/8 AA, 5/8 NA, 7/10 AC, 0/4 NC patients. AID mRNA was detected in biopsies from asthmatics only (5/8 AA, 4/8 NA, 0/10 AC, 0/4 NC). Similarly, CT (Iε-Cγ and/or Iε-Cμ) were observed in biopsies from asthmatics only (6/8 AA and 6/8 NA) and none of the non-asthmatic controls, providing evidence in the former group of ongoing CSR to IgE. Furthermore, we have observed mature message for IgE protein in 6/8 AA, 6/8 NA, 1/10 AC, and 1/4 NC patients. The preliminary results show that the mature IgE mRNA corresponds to the secreted form of IgE.

CONCLUSIONS: This is the first investigation of class switching to IgE in the bronchial mucosa per se. The presence of AID and CT is consistent with CSR occuring locally in the target organ and further that switching may occur directly or sequentially in the mucosa from μ to ε via γ. This may provide the source of locally produced IgE that leads to an immediate reaction after allergen challenge. These findings support local CSR to IgE in the target organ as a potential target for topical therapeutics.

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Mast Cells Progenitors are Recruited to Lung following Induction of Allergic Inflammation and this is Mediated by α4 Integrins, CXCR2 and VCAM-1.

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Homing of mast cell progenitors (MCp) to the intestine under basal conditions is regulated by the CXCR2 chemokine receptor and α4β7 integrin binding to mucosal addressin cellular adhesion molecule (MAdCAM)-1 and vascular cellular adhesion molecule (VCAM)-1 with no role for ²1 integrins. Although the lung in the mouse lacks appreciable baseline homing of MCp, the appearance of increased numbers of mature MC is a characteristic of allergen-induced, TH2 driven pulmonary inflammation in sensitized and challenged mice. We hypothesized that such pulmonary inflammation would be associated with recruitment of MCp by adhesion pathways that are inducible as a consequence of the Th2 response. Mice sensitized with ovalbumin (OVA) adsorbed to alum and challenged with aerosolized OVA had a 3 fold increase in the MCp concentration (per 10^6 mononuclear cells) and a 28 fold increase in the total number of MCp in the lungs after three daily aerosol challenges with further increased numbers after 5 or 7 daily exposures. Mice lacking CXCR2, β7 integrins or endothelial-VCAM-1 exhibited 60%, 72% and 93% reductions respectively in allergen-induced pulmonary MCp (per lung) relative to the wild-type controls. Furthermore, monoclonal antibodies to VCAM-1 inhibited the increase in MCp in the lung by 76% whereas mAb to another endothelial ligand, MAdCAM-1, had no effect. Monoclonal Abs to the VCAM-1 receptors, α4, β1 or β7 integrins, inhibited the increase in the absolute number of MCp in the lung by 69%, 63% and 33%, respectively, in BALB/c mice and by 80%, 80% and 66% respectively, in C57BL/6 mice, while anti-αE did not affect MCp recruitment. These data demonstrate that MCp use both the α4β1 and α4β7 integrins to interact with VCAM-1 for their incremental recruitment to the lung during Th2-biased inflammation, although these numbers suggest that α4²1 integrin is more important than α4²7 integrin. Furthermore, CXCR2 appears to be important in the activity of these integrins in order to allow the inflammation-induced recruitment. Thus, there are both similarities and important differences between the basal homing of these cells to the intestine and the recruitment of these cells to the lung following allergic inflammation.

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Evaluation of the Function of Mast Cell Tryptases using Recombinant Material and Novel Transgenic Mice

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hTryptase beta is a major constituent of the secretory granules of human mast cells (MCs), and this serine protease is stored in the cells as a tetramer ionically bound to heparin-containing serglycin proteoglycans. The mouse ortholog of hTryptase beta is MC protease 6 (mMCP-6; GenBank GeneID 17229). Analysis of the heparin-containing "test cells" in the *Ciona intestinalis* sea squirt revealed the presence of an uncharacterized, enzymatically active serine protease that is ~75% identical to mMCP-6 and hTryptase beta. Sea squirts lack IgE. Thus, our sea squirt data suggest that tryptase/heparin-expressing MCs initially appeared in evolution to control innate immunity rather than adaptive immunity. Two experimental approaches were used to begin to determine the functions of mMCP-6 and hTryptase beta in normal and diseased mammals. In the first approach, both recombinant proteases were generated to evaluate their ability to alter gene expression in different populations of cultured cells. In the second approach, a novel mMCP-6-null mouse strain was created in which the Cre recombinase cDNA was inserted immediately downstream of the translation-initiation site of the mMCP-6 gene. The generated mouse strain should give valuable insight as to why the mMCP-6/hTryptase beta gene has been conserved for >700 million years. Targeted disruption of the mMCP-6 gene did not adversely alter the expression of the other granule proteases in mouse MCs. Thus, our mMCP-6-null mice can now be used to determine the role of this serine protease in any MC-dependent reaction (e.g., sepsis and allergic inflammation). A MC-specific promoter has not yet been created. MCs are the only cells in the animal's body that express mMCP-6. Because of where we placed the Cre gene in our targeting construct, our mMCP-6-null mice also can be used in a Cre-loxP system to selectively alter the expression of any gene in mouse MCs which, in turn, finally allows investigators the opportunity to determine the relative MC-contribution of their favorite gene in a disorder or biologic process in a living animal.

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Integrating control of mast cell function and allergic responses

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Molecular signals are required for control of mast cell homeostasis and activation. Dysequilibrium of these molecular controls can cause detrimental outcomes that may manifest as disease. Thus, regulatory events preceding and following engagement of the high affinity IgE receptor (FcεRI) are likely determinants of the responsiveness of a mast cell. Herein, we identify, and investigate the function of, some molecular regulators of mast cell responsiveness. Among the regulators that control the quiescent state of mast cells, lipid rafts and the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) function as "gatekeepers" of mast cell activation. Genetic downregulation of cholesterol biosynthesis or of PTEN expression results in the activation of PI3K signals and in cytokine production in the absence of degranulation. Antigen-induced activation of mast cells that are deficient in cholesterol or have reduced PTEN expression caused a hyperresponsive phenotype for degranulation and cytokine production with increased activity of multiple signaling pathways. Cholesterol-depletion results in a selective loss of the Src protein tyrosine kinase (Src PTK) Lyn from lipid rafts leading to increased Fyn kinase activity and a hyper-responsive mast cell. Thus, Lyn plays an important role in negatively controlling the extent of antigen-independent and -dependent mast cells responses. In contrast, the activity of another FcεRI-proximal Src PTK, Fyn, was demonstrated to drive and enhance mast cell degranulation and cytokine production. The cellular role of these kinases as negative and positive regulators of a mast cells response is recapitulated *in vivo* in anaphylaxis models. Additionally, generation of sphingosine-1-phosphate, an autocrine regulator of mast cell chemotaxis and degranulation, is controlled by the FcεRI-proximal Src PTKs, Fyn and Lyn. The collective impact is exquisite control of mast cell homeostasis and activation. Herein, these regulatory steps are integrated in the context of IgE-dependent mast cell activation and allergic responses.

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RabGEF1 is a negative regulator of Ras signaling and FcεRI- or c-Kit-dependent activation in mast cells *in vitro*, and of mast cell-dependent biological responses *in vivo*.

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We are attempting to identify endogenous proteins which can limit intracellular signaling and mediator production by mast cells, and to understand the biological significance of such negative regulation of mast cell activation.

Mast cell activation induced by the aggregation of FcμRI with IgE and antigen is the best studied mechanism through which mast cells express immunologically-specific function in health and disease. The dimerization of c-Kit by its ligand, stem cell factor (SCF), is a major mechanism

controlling mast cell development, survival and proliferation, and can also induce mast cells to secrete mediators. We reported that RabGEF1 (Rab guanine nucleotide exchange factor-1, or Rabex-5) binds to Ras and negatively regulates Ras activation and downstream effector pathways during FcμRI-dependent mouse mast cell activation (S. Tam *et al*. *Nat. Immunol*. **5**:844, 2004). Moreover, mast cells derived from RabGEF1-deficient (*Rabgef1*$^{-/-}$) mice exhibited significantly enhanced degranulation, release of lipid mediators and secretion of cytokines in response to FcμRI aggregation *in vitro*. *Rabgef1*$^{-/-}$ mice have increased perinatal mortality and the mice that do survive develop severe skin inflammation and increased numbers of mast cells in the dermis, some of which exhibit morphological evidence of degranulation. These mice also show elevated concentrations of serum histamine and IgE.

We now report that *Rabgef1*$^{-/-}$ mast cells exhibit significantly enhanced levels of IL-6 production in response to stimulation with SCF. Moreover, when *Rabgef1*$^{-/-}$ mast cells are transferred to c-*kit* mutant, genetically mast cell-deficient mice, they survive and, when compared to engrafted populations of *Rabgef1*$^{+/+}$ (wild type) mast cells, can orchestrate higher levels of tissue swelling associated with IgE-dependent passive cutaneous anaphylaxis (PCA) reactions or certain contact hypersensitivity (CHS) responses, and can more effectively enhance the migration of Langerhans cells from sites of hapten sensitization.

Thus, in mast cells, RabGEF1 is a negative regulator of Ras signalling and of both c-Kit- and FcμRI-dependent activation *in vitro,* and, after transfer to c-*kit* mutant mice, *Rabgef1*$^{-/-}$ mast cells can exhibit enhanced mast cell-dependent activity *in vivo* in at least three settings: orchestration of IgE-dependent PCA reactions, enhancement of certain CHS responses and promotion of LC migration at sites of hapten challenge.

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Syk, but not SHIP1, Expression Regulates IgE-mediated Responsiveness of Human Basophils

Donald MacGlashan

Several recent studies in mice have suggested that SHIP (SH2-containing 5' inositol phosphatase) is an expression-modulated element that determines the level of secretion of a mast cell. Studies using human basophils classified as "non-releasers" have suggested that syk kinase expression is a critical element in determining the outcome of the cells' response. We have examined expression profiles of a number of early signaling molecules and related expression of these molecules to two independent parameters of the basophil response. Peripheral blood basophils were assessed for maximum histamine induced by anti-IgE antibody and assessed for basophil sensitivity –the number of antigen-specific IgE molecules per cell required for 50% of the maximum release, ED50, using a new technique combining flow cytometry and traditional histamine release. Basophils purified to near homogeneity, were lysed and syk kinase, lyn kinase, SHIP1, SHIP2, p85 (of PI3 kinase) and Cbl expression measured by standard-calibrated Western blots. For 28 subjects, it was found that alone, syk expression is correlated to function. As a predictor of maximum histamine release, the Pearson R was 0.63 (compare to 0.76 for noise-adjusted median R for a perfect predictor). SHIP1 expression was not correlated. However, in a multiple regression of syk and SHIP1 against maximum histamine release, SHIP1 did inversely account for some variance (approximately 25% of the variance accounted for by syk) suggesting a very modest down-regulatory role. The stimulation index of SHIP1 phosphorylation is also not correlated to maximum histamine release. Neither syk or SHIP1 expression showed a correlation with ED50. Additional studies of a role for SHIP1 suggest that it does not account for the phenomenon of nonspecific desensitization, as might have been expected from signaling kinetic studies. Prior activation, even with a non-IgE dependent stimulus, leads most strongly to down-regulation of syk, with only a weak down-regulation of lyn kinase and a longer-term weak down-regulation of FcεRI. A number of other signaling molecules, including SHIP1, do not change expression with activation. Therefore, the primary regulator of secretion in human basophils appears to be syk kinase and its post-translational control of expression can be mediated by prior activation of the cells.

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Gene expression profiling of human mast cell lines

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Rationale: Although human mast cell (MC) lines, i.e., LAD2 and HMC-1 are widely used, their global profiles of expressed molecules are not reported. In the present study, we analyzed our microarray data of these MC lines in comparison with MC subtypes derived from several human tissues.

Methods: We used Affymetrix GeneChip U133A and hierarchical clustering method to examine the gene expression profiles of MCs derived from several tissues and MC lines. FcεRIα was transfected to HMC-1 with a retrovirus vector. They were then activated through aggregation of FcεRIα.

Results: When the global gene expression of LAD2 was compared with that of MC subtypes and other granulocytes including basophils, LAD2 was classified as a MC subtype. LAD2 exerted a gene expression profile relatively similar to tonsil MCs compared to lung MCs. When MC lines and MCs were compared, however, the differentially expressed genes were divided into the two large clusters, i.e., MC-preferential and MC line-preferential. A small gene cluster consisting of ~200 FcεRIα-mediated upregulated genes containing *IL3*, *IL8* and *AREG* in MCs were identified. The FcεRIα-activated MC lines almost failed to express this gene cluster especially when they were continuously cultured for several months. On the contrary, these cell lines expressed considerable levels of *CCL3*, *CCL4* and *CD69* transcripts which were also markedly upregulated in FcεRIα-activated MCs even after a prolonged culture period. Gene ontogeny term information revealed that genes related to protein biosynthesis and protein folding were markedly upregulated in MCs. On the other hand, MC lines preferentially expressed the genes related to cell cycle, DNA replication and mitosis. In addition, LAD2 and HMC-1 preferentially expressed genes related to G-protein coupled receptor protein signaling and melanoma antigen genes, respectively.

Conclusion: Although human MC lines are extremely useful in terms of availability, several hundred genes are differentially expressed in MCs and MC lines. We should understand such differences to interpret the results using the MC lines appropriately.

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Positive Phadiatop infant® at 2 years increases the risk of asthma at ten years

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Background: Allergic sensitisation in early life is related to later asthma. Phadiatop infant® (Pharmacia diagnostics AB, Uppsala, Sweden), a qualitative marker of allergic sensitisation in young children consists of a panel of specific IgE levels to prevalent allergens. It has not previously been assessed as a quantitative marker for later asthma.

Objectives: To assess if Phadiatop infant® in 2 year old children predicts asthma at ten years.

Methods: In a nested case-control study within the Environment and Childhood Asthma birth cohort study, Phadiatop infant® was analysed by the CAP technique in 368 two year old children (59% with recurrent bronchial obstruction) re-examined at 10 (9-12) years of age for asthma and current asthma. Asthma ever was defined by minimum two of three criteria: obstructive airway symptoms ever, doctor's diagnosis of asthma ever and/or use of asthma medication ever. Current asthma (CA) was defined as asthma ever plus 1 of 3 criteria: obstructive airway symptoms, use of asthma medication in the last 12 months, or positive exercise test.

Results: At age 10, children with CA had significantly higher levels of Phadiatop infant® levels at 2 years (1.97 PAU/L (0.72 -3.22) (mean (95% C.I.)) compared to asthma ever (1.61 PAU/L (0.60-2.61)) and no asthma ever (0.23 (0,17–0,30)) ($p<0.01$). A positive Phadiatop infant® (>0.35 PAU/L) increased the risk of CA (OR: 2.65 (1.46-4.81)($p=0.001$)) and asthma ever 2.8 (1.5-5.0) ($p=0.001$). The Phadiatop infant® at two years had positive and negative predictive values for CA at 10 years of 42.6% and 80.3%, respectively.

Conclusions: Increased levels of Phadiatop infant® at two years significantly increased the risk of CA and asthma ever in children 10 years old. Quantitative analysis of Phadiatop infant® may be useful in risk assessment of later asthma in young children.

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The Impact of Inflammation and Angiogenesis on Airway Mucosal Blood Flow in Asthma

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Background: Increased airway mucosal blood flow (AMBF) and subepithelial vascular engorgement have been demonstrated in asthma and are associated with inflammatory airways disease, angiogenesis and microvascular remodelling. Vascular endothelial growth factor (VEGF), a potent multifunctional cytokine, is central to promoting the process of angiogenesis and acute airway inflammation. We postulated that up-regulation of either airway angiogenesis or inflammation underlies the increase in AMBF found in asthma.

Methods: To test this, airway inflammation (assessed by exhaled nitric oxide [NO ppb] levels) and airway tissue vascularity (CD31 expression in endobronchial biopsy tissue) were correlated with changes in AMBF (assessed non-invasively by soluble gas dilution) in six healthy controls and eight inhaled steroid naïve asthmatics. AMBF is expressed as % acetylene up take in 100mls anatomical deadspace.

Results: See table 1.

Table 1: The relationship between AMBF, inflammation and angiogenesis

Table 1: The relationship between AMBF, inflammation and angiogenesis

	AMBF ($\%C_2H_2$ up take)	FEV_1 (% Predicted)	eNO (ppb)	CD31(+) (% vascularity)
Asthma	18.49 * (+/- 2.25)	87.88 *§ (+/- 3.22)	18.19 *¶ (+/- 3.62)	2.86 *‡ (+/- 0.82)
Healthy	9.68 (+/- 1.68)	106.3 (+/- 2.87)	9.66 (+/- 0.47)	0.85 (+/- 0.33)

Data expressed as mean (+/- SEM) (*p<0.01)

AMBF was higher in asthmatic subjects compared to healthy controls (p<0.01). § AMBF was negatively correlated with FEV_1 % pred values (p<0.01,r=-0.70), ¶positively correlated with exhaled NO (p<0.01, r=0.68) and ‡ % vascularity (p<0.001, r=0.76).

Conclusions: AMBF appears to be a sensitive non-invasive technique for measuring the degree of asthma severity.

AMBF varies with airway calibre perhaps due to changes in bronchial smooth muscle tone resulting from airway hyper-responsiveness or the presence of vascular changes in the remodelling airway.

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Hospital admission with acute asthma exacerbation, specific IgE quantification and virus infection

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Background: Asthma exacerbation is the most common cause of hospital admission in children. We aimed to investigate whether the level of IgE antibodies to inhalant allergens is associated with an increased risk of asthma hospitalization in childhood.

Methods: Children (n=84; age 3-17 years) hospitalized over a one-year period with an acute asthma exacerbation (AA) were matched (age, sex) with 2 controls: stable asthmatics (SA) and children hospitalized with non-respiratory conditions (IC). All subjects underwent measurement of specific IgE (mite, cat, dog; ImmunoCAP™) and nasal lavage for common respiratory viruses and atypical bacteria (PCR).

Results: A significantly higher proportion of AA had a respiratory pathogen detected (44%) compared to SA (18%) and IC (17%; p<0.001). Similarly, sensitization (IgE> 0.35 kU_A/l) was significantly more common amongst AA than SA and IC groups (90% vs. 65% vs. 33% respectively; p<0.001). Further analysis of risk factors for hospital admission was carried out within the two groups of asthmatic patients (AA and SA) using logistic regression. Using individual specific IgE levels as a continuous variable, the risk of admission increased significantly only with increasing IgE to mite (OR 1.2, 95% CI 1.1-1.4, p=0.004). However, when specific IgE levels to mite, cat and dog were summed, the probability of hospitalisation increased 1.4-fold (95% CI 1.2-1.7, p<0.001) per logarithmic unit increase in IgE (Figure 1). Furthermore, even among sensitized children, the sum of mite, cat and dog specific IgE significantly increased the risk of hospitalization (1.35, 1.0-1.8, p=0.04) (Figure 2.)

Figure 1.

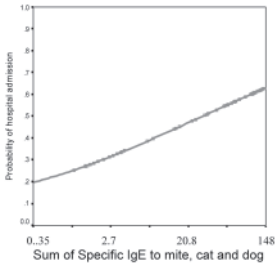
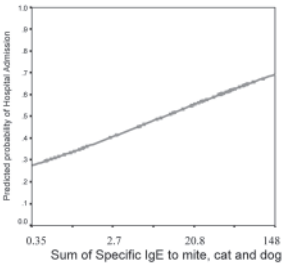


Figure 2.



In the multivariate regression analysis, the sum of mite, cat and dog specific IgE remained a significant and independent associate of hospital admission (1.26, 1.02-1.56, p=0.03). In addition, there was a significant interaction between the sum of IgEs and virus infection in increasing the risk of admission (1.59, 1.01-2.5, p=0.04)

Conclusions: Increasing specific IgE antibody levels interact with natural virus infection in increasing the probability of hospitalization amongst childhood asthmatics.

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Tumor necrosis factor-related apoptosis inducing ligand is a key regulator of Th2 cell responses and allergic disease of the lung

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Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) selectively induces apoptosis in tumor cells but little is known about its role in normal, non transformed tissues. We report here that TRAIL is abundantly expressed in the airway epithelium of allergic mice. RNA interference mediated targeting of TRAIL results in reduced Th2 cytokines (IL-13, IL-4, IL-5), signal-transducer-and-activator-of-transcription 6 (STAT6) expression and allergic airway inflammation. In the absence of TRAIL the development of airways hyperreactivity (AHR) is completely abolished. Recombinant TRAIL delivered to the lungs induces AHR, airway inflammation and IL-13 release in naive wild type, but not IL-13 deficient mice. Thus, TRAIL is both essential and instructive in the development of AHR by activating STAT6 mediated effector functions. Suppression of TRAIL expression in the airway epithelium may be therapeutic in bronchial asthma.

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Reversing the defective induction of interleukin-10 secreting T cells in glucocorticoid resistant asthma patients.

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A proportion of asthmatic patients fails to benefit from oral glucocorticoid therapy and is denoted as having glucocorticoid resistant or insensitive asthma (SR). SR is associated with in vivo and in vitro alterations in cellular responses to exogenous glucocorticoids. We have demonstrated that CD4+ T cells from SR asthma patients fail to induce IL-10 synthesis following in vitro stimulation in the presence of dexamethasone, as compared with their glucocorticoid sensitive asthmatic counterparts, suggesting a link between induction of IL-10 synthesis and clinical efficacy of glucocorticoids. We now investigate whether this defect in the induction of IL-10 synthesis is reversible both in vitro and in patients.

We have previously reported that human CD4+ T cells secrete high levels of IL-10 when stimulated in the presence of dexamethasone and calcitriol (vitamin D3), as measured by quantitative PCR, ELISA and intracellular cytokine staining. We now show that following stimulation by allergen, these drug-induced cells inhibit cytokine secretion by allergen-specific Th2 cells, a major therapeutic target in allergic disease, in an IL-10-dependent manner. Addition of vitamin D3 with dexamethasone to cultures of SR CD4+ T cells enhanced IL-

10 synthesis to levels observed in cells from glucocorticoid sensitive patients cultured with dexamethasone alone. Furthermore, pre-treatment with IL-10 fully restored IL-10 synthesis in these cells in response to dexamethasone. Vitamin D3 significantly overcame the inhibition of glucocorticoid receptor expression by dexamethasone, whilst IL-10 upregulated glucocorticoid receptor expression by CD4+ T cells, suggesting potential mechanisms whereby these treatments overcome poor glucocorticoid responsiveness. In a proof of concept study we administered oral vitamin D3 every day for one week at a standard clinical dose, to both healthy subjects and SR asthma patients and demonstrated that glucocorticoid-induced IL-10 mRNA and protein was enhanced in all individuals following this treatment.

Our findings suggest that vitamin D3 has the potential to increase the therapeutic response to glucocorticoids in SR patients, a patient group where therapeutic options are limited.

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IgE against Staphylococcus Aureus enterotoxins and asthma severity

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The pathophysiological mechanisms responsible for severe and persistent asthma are not well understood. *Staphylococcus aureus* derived enterotoxins (SAEs) are a group of superantigens (SAgs) that have been implicated in the pathogenesis of chronic inflammatory diseases and elevated specific IgE against SAE's have been described in atopic eczema and in nasal polyposis, both conditions associated with tissue eosinophil infiltration. To investigate whether the presence of IgE directed against SAEs was related to severity of asthma, cohorts of non-asthmatic controls (n=49), mild asthmatics (British Thoracic Society [BTS] management step 1, n=55) and severe asthmatics (BTS management steps 4/5, n=52) were recruited and characterized. The concentrations of total IgE, specific IgE against house dust mite (HDM) and cat allergens, specific IgE to SAEs (reflected by an assay measuring the mix of antibodies against SAE's A, C, and TSST-1) and the leucocyte activation markers eosinophil cationic protein (ECP) and myeloperoxidase (MPO), were measured in serum by ELISAs.

Specific IgE to HDM and cat allergens were raised in mild asthma, in comparison to both controls and severe asthma, whereas no significant difference existed between the healthy controls and the severe asthmatics in these measures. By contrast, the presence of IgE against SAEs was found to be related to asthma severity, with 8/49 (16.3%) controls having IgE to SAEs compared to 19/55 (34.5%) of mild asthmatics, and 28/52 (53.8%) of severe asthmatics (differences $p<0.05$ for each step up in severity). Within each group total IgE levels were significantly higher in patients with IgE to SAEs in comparison to those without, consistent with polyclonal SAg stimulation. Within the severe group, aspirin sensitivity, late onset asthma, and male sex were significantly related to the presence of IgE to SAEs. In comparison to the healthy controls, serum ECP was significantly raised in both groups of asthmatics but these measures did not distinguish between mild and severe asthma. No differences existed between groups in serum MPO.

These results suggest the potential importance of IgE synthesis related to bacterial products and in particular to SAE, rather than to classical aeroallergens, in the pathogenesis of severe asthma.

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Involvement of periostin in subepithelial thickening of bronchial asthma downstream of IL-4 and IL-13 signals

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Thickening of the reticular basement membrane is a cardinal feature of bronchia asthma. A lot of evidence supports that it is correlated with the clinical and functional severity of bronchial asthma. Extracellular matrix proteins, such as collagens I, III, and V, fibronectin, and tenascin-C, but not collagen IV and laminin, are deposited in the thickened reticular basement membrane. However, not all of its deposited components has been characterized and it remains obscure how these components generate the reticular structure. IL-4 and IL-13, Th2 cytokines sharing receptors and signal pathways, have a pivotal role in this process, which is assumed to be dependent on induction of TGF-β. However, it is unclear whether subepithelial thickening by IL-4 and IL-13 is explained by the TGF-β–dependent pathway alone. We have previously identified the *POSTN* gene encoding periostin as an IL-4/IL-13–inducible gene in bronchial epithelial cells by the microarray approach. Periostin is thought to be an adhesion molecule because it possesses four fasciclin I domains, characteristic of a family of adhesion molecules. In this study, we explore the possibility that periostin is involved in subepithelial thickening in bronchial asthma. Both IL-4 and IL-13 induced secretion of periostin in lung fibroblasts independently of TGF-β. Periostin co-localized with other extracellular matrix proteins involved in subepithelial thickening in both asthma patients and asthma-induced wild mice, but not in either IL-4 or IL-13 knockout mice. Periostin had an ability to bind to fibronectin, tenascin-C, collagen V, and periostin itself, but not to collagens I and III. Periostin secreted from lung fibroblasts by IL-4 and/or IL-13, assumes to be involved in subepithelial thickening in bronchial asthma, interacting with other extracellular matrix proteins. These results demonstrate that TGF-β–dependent and –independent pathways co-operate with each other, generating subepithelial thickening of bronchial asthma, and indicate that IL-4/IL-13 signals would be a promising target to inhibit this process.

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Expression and function of thymic stromal lymphopoietin (TSLP) in allergic rhinitis patients

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Background: Accumulation of activated Th2 cells is believed to play a pivotal role in the immunopathology of allergic reactions. The mechanisms that regulate the activation of these T cells are, however, poorly understood. Recently, TSLP was reported to potently activate classical myeloid dendritic cells (MDC) to prime naive T cells to produce the Th2 cytokines IL-4, IL-5, and IL-13. However, whether TSLP is involved in the activation of Th2 memory cells has not been determined. Because MDC are the major DC subset in the upper airway mucosa, we examined the expression and function of TSLP related to Th2 memory responses in allergic rhinitis patients.

Methods: Mucosal biopsies from patients with allergic rhinitis and non-atopic controls, obtained before and during local allergen challenge, were subjected to multi- immunofluorescence staining. MDC and CD4+ T cells purified from peripheral blood of allergic patients and controls were co-cultured with or without allergen and TSLP. T-cell activation was measured by proliferation and cytokine production.

Results: The addition of TSLP in MDC/T-cell co-cultures dramatically increased allergen-dependent proliferation as well as production of IL-5 and IL-13, whereas levels of IFN-γ were unchanged. Importantly, a TSLP-DC:T-cell ratio of 1:150 induced higher IL-5 and IL-13 levels than the optimal MDC:T-cell ratio (1:10) without TSLP. The costimulatory molecule CD86 was upregulated on TSLP-DC and the TSLP-mediated effect on T-cell activation was partly blocked by anti-CD86, but not by anti-CD80 or anti-Ox40L. TSLP protein was expressed in the nasal epithelium of both allergics and non-allergics. The expression appeared to be constitutive and restricted to the nasal transitional epithelium. After local allergen challenge an increased number of MDC was observed in the nasal mucosa concomitantly with accumulation of activated (CD25+) T cells and eosinophils, but only in the allergic patients.

Conclusion: We show here that TSLP-activated MDC strongly augment allergen-specific Th2 responses and that MDC accumulate in parallel with activated T cells in experimentally-induced allergic rhinitis. The observation that TSLP is expressed in the nasal epithelium of these patients indicates that TSLP is directly involved upper airway allergy.

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Essential role of Interferon-γ and Th1 immune response in the pathogenesis of non-eosinophilic asthma

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Asthma is defined by a chronic inflammatory disorder of airways which is associated with reversible airway obstruction and airway hyperresponsiveness (AHR). Th2 cytokines such as IL-4 and IL-13 cause airway inflammation characterized by eosinophil infiltrations and airway remodeling which has long been regarded as a hallmark of asthma.

However there has been accumulating evidence that prominent bronchial neutrophilia occurs in situations associated with severe asthma.

Our study revealed that less than half of adult asthma patients show sputum eosinphils and this is not associated with asthma severity. On the other hand, it was found that sputum neutrophils and INF-γ mRNA expression are increased in severe asthma patients. Moreover lung-specific INF-γ transgenic (TG) mice were found to show markedly enhanced AHR along with lung inflammation characterized by the infiltrations of macrophages, lymphocytes, and neutrophils.

It is well known that episodic exacerbations of asthma are mostly associated with viral or bacterial infections. With these infections, innate immune system could be activated by recognizing the pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and which subsequently modulate achaptive immunity secreting IL-12 from dendritic cell and producing Th1 cell from naïve T cell.

We demonstrated that chronic airway challenge to allergen with LPS enhances AHR and airway inflammation infiltrating with macrophages, lymphocytes and neutrophils when compared with allergen alone in animal model. Furthermore airway sensitization of an allergen along with dsRNA (a byproduct of viral replication) induced Th1 cell priming, enhanced AHR, non-eosinophilic airway inflammation, and allergen-specific IgG2a production, and these were inhibited in the absence of INF-γ.

These findings suggest that INF-γ and Th-1 immune response play the key role in the pathogenesis of non-eosinophilic asthma.

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Allergen-induced asthma-like reaction in sensitized guinea pigs was reduced by pretreatment with epigallocatechine-3-gallate

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Asthma is a common inflammatory airway disease whose prevalence is ever-increasing, and whose major pathophysiological hallmarks are mast cell activation, increased endothelial expression of adhesion molecules and enhanced leukocyte recruitment. It has been showed that nitric oxide (NO) plays a role in asthma, even if the pathophysiological connection between NO and asthma remains uncertain. In this study we used an animal model of asthma-like reaction, to provide insight into the possible role of NOS-derived NO in the pathophysiology of early asthma and to test the possible therapeutic effect of epigallocatechin-3-gallate (EGCG), a polyphenol that enhances NO synthase (NOS) activity. For comparison, we used epicatechine (EC), which shares antioxidant but not NOS-modulating properties with EGCG.

Ovalbumin-sensitised guinea pigs placed in a respiratory chamber were challenged with ovalbumin. EGCG (25 mg/kg b.wt.) or EC (25 mg/kg b.wt.) were given i.p. 20 min before ovalbumin challenge. We analysed latency time for the onset of respiratory abnormalities; cough severity; duration of dyspnea; lung tissue histopathology; mast cell activation (by granule release); leukocyte/eosinophilic infiltration (by major basophilic protein, eMBP, and myeloperoxidase, MPO); oxygen free radical-mediated injury (by nitrotyrosine, NT, and 8-hydroxy-2-deoxyguanosine, 8OHdG); NOS activity; bronchial inflammatory response (by TNF-α in bronchoalveolar lavage, BAL).

Severe respiratory abnormalities appeared in the sensitised animals soon after the antigen challenge, accompanied by bronchoconstriction, alveolar inflation and a marked increase in the assayed parameters of inflammatory cell recruitment, free radical lung injury and release of proinflammatory molecules in BAL fluid. This was associated with marked depression of constitutive NOS activity. Pretreatment with EGCG, but not EC, significantly reduced all the above parameters and sustained endothelial-type NOS activity.

These findings indicate that EGCG, probably by modulating NOS activity, can counteract allergic asthma-like reactions in sensitised guinea pigs and suggest that it may be useful for the treatment of asthma in the future.

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Interaction of Allergic Rhinitis and Bacterial Sinusitis in a Mouse Model

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University of Chicago

Background: We previously showed that when BALB/c mice with an ongoing allergic reaction become infected, they develop an augmented bacterial and inflammatory response in the paranasal sinuses on day 5.

Objective: To study the importance of long-term allergen exposure and of a TH1/TH2 genetic background in augmenting bacterial and inflammatory responses in allergic and infected mice.

Methods: BALB/c and C57BL/6 mice were given intraperitoneal injections of OVA or PBS followed by intranasal OVA or PBS challenge. After 1 day of intranasal allergen exposure, they were inoculated intranasally with *Streptococcus pneumoniae*. The numbers of bacteria and inflammatory cells in the sinuses 5, 14, 21, and 28 days after infection were measured. Nasal responsiveness to histamine, a marker of hyperresponsiveness, was assessed 2 days before the first sensitization and on days 3, 12, 19, and 26 after sensitization.

Results: BALB/c and C57BL/6 mice that were OVA-sensitized, OVA-challenged, and infected showed significantly more bacteria and phagocytes than did nonsensitized, OVA-challenged and infected mice from days 5 to 28 after infection. These differences were diminished after the allergen challenge was stopped, suggesting it is the active inflammation that augments the bacterial infection. Although C57BL/6 mice can be made allergic, they showed fewer bacteria, eosinophils, and phagocytes than did allergic and infected BALB/c mice. Surprisingly, in contrast to C57BL/6, nonallergic, infected BALB/c mice showed a significant number of bacteria at 28 days after infection.

Conclusion: Long-term ongoing allergic reaction augments bacterial infection in both BALB/c and C57BL/6 mice and induces nasal hyperreactivity to histamine. However, allergic and infected C57BL/6 mice show less allergic inflammation and infectious responses than do allergic and infected BALB/c mice. Stopping allergen exposure reduces the allergic response and the augmented response to infection. Infected BALB/c mice, which favor a TH2 response, were less able to clear infection than were C57BL/6, which favor a TH1 response. Allergic inflammation and augmented infectious response are affected by the genetic background of mice and by ongoing allergen stimulation.

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Role of IL-5, eosinophils and TGF-β1 in allergen-induced subepithelial fibrosis in mice

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Asthma is a chronic inflammatory disease characterized by variable bronchial obstruction, airway hyperresponsiveness and by tissue damage known as airway wall remodeling. In the present study we demonstrate that eosinophil plays an obligatory role in the airway remodeling observed in experimental asthma. BALB/c mice sensitized by intraperitoneal injections of ovalbumin (OVA) and exposed daily to aerosolized OVA for up to 3 weeks, develop eosinophil infiltration into the bronchi and subepithelial and peribronchial fibrosis. The lesions are associated with increased amounts of hydroxyproline in the lungs and elevated levels of eosinophils and TGF-β1 in the bronchoalveolar lavage fluid. After one week of allergen challenge, TGF-β is mainly produced by eosinophils accumulated in the peribronchial and perivascular lesions. At a later stage of the disease, the main source of TGF-β is myofibroblasts, identified by ±-smooth muscle actin monoclonal antibody. We show that all these lesions, including fibrosis, are abolished in sensitized and allergen exposed IL-5 receptor ±-chain gene deficient mice, whereas they are markedly accentuated in IL-5 transgenic animals. More importantly, treatment of wild type mice with neutralizing anti-IL-5 antibody, administered before each allergen challenge, almost completely prevented subepithelial fibrosis. Furthermore, a neutralizing antibody against TGF-β1 markedly attenuated allergen-induced subepithelial fibrosis by the administration between day 22 and 31, whereas it inhibited the development of suepithelial fibrosis by the administration between day 32 and 42 model. These findings demonstrated that eosinophils are involved in allergen-induced subepithelial fibrosis by producing a fibrogenic factor, TGF-β1.

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Induction and efficacy of cytolytic regulatory T cells in experimental asthma

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Inducing regulatory T cells (Tregs) represent an attractive therapeutic strategy for allergic asthma, as they require cognate interaction with a specific peptide for activation while exerting intra- or even inter-molecular suppression.

Cytolytic CD4+ Tregs (J. Immunol. 171:4604, 2003) can be generated under specific conditions in animals and expanded in vitro. Such Tregs express a $CD25^{hi}$ $CTLA\text{-}4^{hi}$ phenotype but produce very little of the commonly advocated suppressing cytokines, IL-10 and TGF-b, and are therefore distinct from currently described Tregs. Upon activation, they lyse antigen-presenting cells after specific peptide recognition using the Fas/FasL and/or the granzyme/perforin pathway, depending on target cell, including dendritic and B cells. Intra- and inter-molecular suppression can readily been demonstrated upon incubation with effector CD4+ T cells of unrelated specificity.

In vivo, induction of cytolytic Treg fully prevents or suppresses both airway hyperreactivity and inflammation in BALB/c mouse experimental asthma induced by peripheral sensitization and inhalation with recombinant Der p 2. Such efficiency is independent of the presence of a functional humoral response as shown in JHD mice, which lack B cells. Treg clones were derived from splenocytes of immunized animals. Transfer of as little as $2x10^5$ cells to mice either prior to or after allergen sensitization fully abrogate the development of airway hyperreactivity and inflammation.

These data show that allergen-specific Tregs can control the two main independent manifestations of asthma, airway hyperreactivity and inflammation. As such, they hold promise for an application in human allergic asthma.

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Nasal Nitric Oxide in Objective Evaluation of Chronic Rhinosinusitis Therapy

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Background -The assessment of the response of chronic rhinosinusitis to therapy is difficult. CT scans cannot be repeatedly used so measures such as symptom scores, endoscopic findings, and parallel measures such as saccharin clearance time are employed instead.

Objective- To study the effect of chronic rhinosinusitis therapy on nasal nitric oxide and to see whether nasal nitric oxide level changes correlate with other assessments.

Methods- The study was a prospective randomized trial of patients with chronic rhinosinusitis, with or without polyps, who had failed initial medical therapy with douching and nasal corticosteroids and who then had abnormal CT scans. They were treated either medically or surgically , with follow up at 6 and 12 months whilst still taking nasal corticosteroids. Nasal nitric oxide was measured initially and at 6 and 12 months as well as symptom scores, endoscopy, polyp grading , saccharin clearance time.

Results- Initial absolute nasal nitric oxide levels correlated inversely with CT scan changes, ($p<0.001$). The percentage rise in nasal nitric oxide seen on both medical and surgical treatment correlated with changes in symptom scores ($p<0.001$), saccharin clearance time ($p<0.001$), endoscopic changes ($p<0.001$), polyp grades ($p<0.05$ at 6months, $p<0.01$ at 12 months) and surgical scores ($p<0.01$) .There was no significant correlation with age, sex, smoking or allergy.

Conclusion- Nasal nitric oxide, which is easily measured, provides a valuable non-invasive objective measure of the response of CRS to therapy. Topical nasal corticosteroids may be needed to reduce the contribution of nasal epithelial nitric oxide and allow that emanating from the sinuses to be measured.

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Pro-angiogenic Properties of Bronchoalveolar Lavage Fluid from Asthmatics

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Rationale: Asthmatic airways exhibit an increase in the number and size of vascular structures, which contribute to airflow obstruction and hyperresponsiveness. We have compared the pro-angiogenic potential of bronchoalveolar lavage fluid (BALf) from asthmatic and healthy subjects.

Methods: 4 mild steroid naive asthmatic, and 4 healthy volunteers were recruited. Angiogenic activity of BALf was evaluated in a co-culture of human umbilical vein endothelial cells and dermal fibroblasts. BALf was concentrated (×40) using a centrifugal filter with 10kD exclusion limit. Vascular structures were visualised by anti-CD31 labelling. Vascular formation was quantified using image analysis. BALf vascular endothelial growth factor (VEGF) levels were determined by ELISA.

Results: Healthy BALf (final concentration ×4) induced the formation of CD31 positive tubular vascular structures that was 3-fold greater than with culture medium alone. Vascular junctions and tubule length were increased 2-fold in the asthmatic group ($p=0.023$ and $p=0.011$ respectively) and the number of vascular tubules increased by 1.7-fold in asthmatic BALf compared to healthy controls ($p=0.02$). VEGF receptor (KDR/flt-1) antagonism with suramin (100μM) reduced all angiogenic indices by 80-90% in both subject groups ($p<0.01$). Likewise, a function blocking VEGF antibody inhibited all vascular indices of vascular tubule formation by 50-80% in healthy ($p<0.05$) and 76-88% in asthmatics ($p<0.01$). VEGF levels in BALf were increased (2.8-fold) in asthmatic subjects compared with healthy subjects ($p<0.024$).

Conclusions: Pro-angiogenic activity (>10kD) is present in BALf from healthy subjects and is elevated in asthmatics. The angiogenic stimulus is mediated via a VEGF dependent pathway.Understanding cellular sources and regulation of angiogenic factors may result in future therapeutic strategies for treating asthma.

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Aspirin sensitive rhinosinusitis is associated with reduced E-prostanoid 2 (EP2) receptor expression on nasal mucosal inflammatory cells

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Patients with idiosyncratic reactions to aspirin ingestion characteristically exhibit the clinical triad of aspirin sensitivity, asthma and chronic polypoid rhinosinusitis.[1] A fundamental feature of aspirin sensitivity is excessive production of cysteinyl leukotrienes (Cys LTs) in the steady state, which is further elevated following aspirin exposure. While certain prostanoids, particularly PGD_2, are regarded as having pro-inflammatory properties and contributing to bronchoconstriction in aspirin sensitive asthma, there is good evidence that the prostanoid PGE_2 inhibits aspirin-induced phenomena. Impaired "braking" of inflammatory cell Cys LT production by prostaglandin E2 (PGE_2) has been implicated in the pathogenesis of aspirin exacerbated airways disease, but the mechanism is obscure. PGE_2 acts via G-protein-coupled receptors, $EP_{1\text{-}4}$, but there is little information on the expression of PGE_2 receptors in this condition. We have now addressed the hypothesis that expression of one or more EP receptors on nasal mucosal inflammatory cells is deficient in patients with aspirin-sensitive, as compared with non-aspirin-sensitive, polypoid rhinosinusitis.

Using specific antibodies, immunohistochemistry and image analysis, we measured the expression of $EP_{1\text{-}4}$ in nasal biopsies from patients with aspirin sensitive (n=12) and non-aspirin sensitive (n=10) polypoid rhinosinusitis and normal controls (n=9). Double staining was employed to phenotype inflammatory leukocytes expressing $EP_{1\text{-}4}$. The results showed that global mucosal expression of EP_1 and EP_2, but not EP_3 or EP_4 immunoreactivity was significantly elevated in aspirin sensitive and non-aspirin sensitive rhinosinusitis as compared with controls ($p<0.03$). This was attributable principally to elevated expression on $tubulin^+$ epithelial cells and $Muc\text{-}5AC^+$ goblet cells. In contrast, the percentages of neutrophils, mast cells, eosinophils and T cells expressing EP_2, but not EP_1, EP_3, or EP_4 were significantly reduced ($p\leq0.04$) in the aspirin sensitive, as compared with non-aspirin sensitive patients. The data suggest a possible role for PGE_2 in mediating epithelial repair in rhinitis and asthma. Since PGE_2 exerts a range of inhibitory actions on inflammatory leukocytes via the EP_2 receptor, its reduced expression in aspirin sensitive rhinosinusitis may be partly responsible for the increased inflammatory infiltrate and production of cysteinyl leukotrienes which characterise aspirin sensitive disease.

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Effects of lipid mediators on the activation of human lung fibroblasts

Shigeru Takafuji

Background: Lipid mediators such as LTC4 and PAF play important roles in the pathogenesis of bronchial asthma. However, the effects of lipid mediators on human lung fibroblasts and the mechanism of eosinophil recruitment by those effects have not been thoroughly elucidated. In this study, we examined effects of LTC4 and PAF on eotaxin production and adhesion molecules expression by human lung fibroblasts.

Methods: Fibroblasts were cultured with chemical mediators including LTC4 and PAF in the absence or presence of IL-4 for 48h. At the end of the culture period, eotaxin in the supernatant was measured by ELISA. In addition, adhesion molecules expression on fibroblasts was analyzed by flow cytometry.

Results: IL-4 clearly enhanced eotaxin production by fibroblasts. LTC4 alone had no effect on eotaxin production. When fibroblasts were cultured with IL-4 plus LTC4, eotaxin production was significantly enhanced in comparison with IL-4 alone. In the presence of IL-4, the enhancement of eotaxin production by LTC4 was concentration dependent, becoming maximal at 10^{-6}M. PAF alone had no effect on eotaxin production. When fibroblasts were cultured with IL-4 plus PAF, eotaxin production was significantly enhanced in comparison with IL-4 alone. Also when fibroblasts were cultured with IL-4 plus PAF, vascular cell adhesion molecule(VCAM)-1 expression was clearly enhanced in comparison with IL-4 alone. Y-24180(PAF antagonist) clearly inhibited PAF effects on eotaxin production and VCAM-1 expression.

Conclusion: These results suggest that lipid mediators such as LTC4 and PAF may increase eotaxin production and VCAM-1 expression by fibroblasts in the presence of IL-4 and that lipid mediators may induce eosinophil recruitment into airways through the activation of lung fibroblasts in the presence of IL-4 in allergic inflammation.

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Epidemiology of the Allergic Respiratory Syndrome in the Residents of Urban US Public Housing Communities

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In the US, low-income urban population is characterized by the highest asthma mortality and morbidity. Public housing residents represent the lowest income group, but the epidemiology of asthma in this group is not known. Our approach to studying asthma is to consider it part of a respiratory syndrome that affects the entire airway. We have conducted a door-to-door population survey of two Baltimore City public housing communities. The survey instrument queries for the presence of upper and lower airway symptoms and for a physician's diagnosis of asthma and rhinitis/sinus disease. An algorithm categorizes participants into "probable" and "possible" asthma (PrA and PosA), no asthma (NA), "probable" and "possible" rhinosinusitis (PrRs and PosRs) and no rhinosinusitis (NRs). We have surveyed 600 individuals representing approximately 50% of the target population. Average age is 30 years, 68% are female and 85% African American. Survey prevalence was as follows: PrA: 21%, PosA:16.9%, PrRs: 50.9%, PosRs: 16.4%. Among the PrA, 79% were categorized as PrRs and only 1% as NRs. The probability of being PrA increased linearly with increased number of rhinosinusitis symptoms (R^2=0.83). Eighty individuals were invited to undergo further evaluation with skin testing or specific IgE measurements and with methacholine bronchoprovocation or albuterol reversibility. On the basis of bronchial hyperresponsiveness (PC_{20} < 8 mg/ml) or FEV_1 reversibility (>12%), approximately half of the PrA and PosA subjects were confirmed as asthmatics. Extrapolation of these data to the entire population yields an asthma prevalence of 21.9%. Allergy testing (12 allergens) comparisons of 20 subjects with confirmed asthma and PrRs/PosRs to 22 with no asthma but with PrRs/PosRs and to 14 with no asthma and NRs, showed that, for every allergen, the prevalence of sensitization increased from the latter to the former group. We conclude that, in this population a) asthma prevalence is 3-fold higher than that of the entire US, b) rhinosinusitis symptoms are ubiquitous in asthmatics and c) the overall degree of atopy differentiates individuals with the fully developed respiratory syndrome from those with upper airway involvement alone.

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Chymase polymorphism is associated with the severity of atopy in asthmatics

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Background: Mast cell chymase has the potential to be an important mediator of inflammation and remodelling in the asthmatic lung. Previous studies have examined associations between promoter polymorphism of the MCC gene (CMA1) and allergic phenotypes but the significance of this polymorphism is unclear. Recently, two rare non-synonymous single nucleotide polymorphisms of CMA1, His45Arg (rs5247) and Gly25Arg (rs5246), have been identified. Modelling studies have shown that these SNPs are likely to be in, or near to, the active site of chymase, and may represent functional genetic variants.

Aim: To investigate the presence of these polymorphisms in a UK Caucasian population and to assess association with asthma and allergy phenotypes in a large family based cohort.

Methodology: UK Caucasian families (n = 336) with ≥2 affected sibs, with a physician's diagnosis of asthma and current medication use, were studied. Non-asthmatic Caucasians (184) without a family history of asthma were recruited as "hyper-normal" controls. Genotypes were determined by tetra-primer ARMS-PCR.

Results: The Arg25 allele was present at a frequency of < 0.005 in the control (non-asthmatic) cohort and was not analysed further. For the His45Arg SNP, the Arg allele was present at a frequency of 0.03 in the control cohort. No association of the His45Arg polymorphism was found with the risk of developing asthma in parent-affected sib trios using the transmission disequilibrium test, or in case (asthmatic)-control analyses using $Ç^2$ (P>0.05). In first affected sibs, both total IgE (P=0.026) and the atopy severity score (size and number of positive skin prick tests and specific IgE) was higher (P=0.001) in the His/Arg or Arg/Arg genotypes compared to the His/His genotype. No associations were found with FEV_1 % predicted, BHR to methacholine or asthma severity score (P>0.05).

Conclusions: These data suggest that a polymorphism in the human mast cell chymase gene coding 867A/G (His45Arg) may influence the regulation of total and specific IgE levels and atopy severity. Further investigation of the effect of this polymorphism on chymase function is warranted.

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Lymphocyte Migration to the Lung

Andrew Wardlaw, Caroline Palmquist, Morgan Angela, Guillen Cristina

Patterns of migration of memory T cells into tissue include organ specific trafficking as part of a homeostatic process of lymphocyte homing and inflammation related migration of either Th1 and Th2 cells depending on the pathogenic insult. T cell migration is controlled in large part by chemokines expressed in an organ or disease specific manner which attract selective subsets of T cells expressing distinct patterns of chemokine receptors (CR). We have characterised the chemokines and CR controlling T cell migration to the lung in normal subjects, asthmatics and patients with interstitial lung disease (ILD). Of a comprehensive panel of CR only expression of CXCR6 was markedly up-regulated on BAL and lung tissue T cells compared to blood T cells. There was no significant difference in the pattern of CR expression on T cells from normal subjects and asthmatics although patients with ILD had increased expression of CXCR6. T cells expressing IL-4 (putative Th2) expressed CXCR6 to the same degree as T cells producing INFγ (Th1). CXCL16, the only ligand for CXCR6 was present in high concentrations in BAL fluid (mean 2000pg/ml) with no increase in inflammatory lung disease compared to normal subjects suggesting it is involved in constitutive migration. CXCL16 was synthesised by alveolar macrophages and bronchial epithelial cells with a modest increase induced by stimulation with INFγ. The preferential expression of Th1 and Th2 related CR, respectively CXCR3/CCR5 and CCR3/CCR4, on BAL derived T cells was also investigated. 5% (+/- 0.1) of BAL T cells released IL-4 and 33% (+/-3.7%) IFNγ after stimulation. The IL-4 producing T cells preferentially expressed CCR3 and CCR4 compared to IFNγ producing Th1 cells. There was a largely non-significant trend towards increased expression of CXCR3 and CCR5 on INFγ as opposed to IL-4 producing T cells. In conclusion CXCR6 and its ligand CXCL16 are potential involved in lung T cell homing. CCR3 and CCR4 binding chemokines may cause preferential recruitment of Th2 cells in to the lung in asthma

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IgE dependent cytokine release from human lung tissue

Tillie Hackett, Jane Warner

The crosslinking of IgE is now known to contribute to chronic inflammation and airway remodelling. We have examined the cascade of cytokines released from human lung tissue following an IgE mediated stimulus. We obtained human lung tissue from 19 patients with an average age of 68±3 years. There were 12M/7F and 3 non-smokers, 6 current smokers and10 ex-smokers. Lung fragments were challenged with a range of different concentrations of anti-IgE (1,000 –0.1 µg/ml) and cytokine release measured 24 hrs later. In a second series of experiments the lung tissue was stimulated with either 100 or 1µg/ml anti-IgE and the release of cytokines followed for up to 48 hrs. IgE mediated cytokine release was compared with 100ng/ml LPS and a buffer control. The release of IL-5 and IL-13 was maximal at 1µg/ml anti-IgE with a bell shaped dose response curve. The amount of IL-5 released was generally low (mean=3.23±1.95 ng/mg tissue compared to 0.28±0.06 ng/mg tissue in the control) The release of IL-13 was also low (mean = 3.96±1.26 ng/mg tissue compared with 1.14±0.35 ng/mg tissue in the control tissue). The release of IL-5 and IL-13 was associated with the IgE dependent stimulus and did not occur in tissue challenged with LPS. In contrast, TNFα, IL-6, IL-8 and IL-10 all peaked at 1000µg/ml anti-IgE. The levels of TNFa reached 93.9±21.0 ng/mg tissue following 1000µg/ml anti-IgE compared with 45.3±7.4 ng/mg tissue when 100ng/ml LPS was used as a stimulus. The levels of IL-6 and IL-8 were much higher reaching in excess of 1000ng/mg tissue for IL-6 and 3,000 ng/mg tissue for IL-8. Detailed analysis of the kinetics revealed that IL-5 and IL-13 were released early (<2hours) while the other cytokines required 24-48 hours to reach a maximum. In summary we have demonstrated that the release of IL-5 and IL-13 occurs within 2 hours of IgE crosslinking and requires much lower concentrations of anti-IgE than other inflammatory cytokines such as TNFα, IL-6 and IL-8.

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Role of advanced glycation end-products in lung disease

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Advanced glycation end-products (AGE) form on long-lived connective tissue and matrix components by a process of non-enzymatic glycosylation. AGE accumulation is a normal ageing process; however it is accelerated in several pathological conditions, for example diabetes mellitus and pulmonary fibrosis. AGE have a number of chemical and biological properties that are potentially pathogenic, including induction of cytokine and growth factor synthesis, increase in vascular permeability, enhancement of cell proliferation and extra-cellular matrix production. This process may therefore play a role in the pathophysiology of asthma. To investigate this we have immunohistochemically stained bronchial biopsies from mild asthmatics and normal controls for AGE. Immunoreactivity was assessed by computerised image analysis in inflammatory cells, the extra-cellular matrix (ECM) and the bronchial epithelium. Expression was increased in the bronchial epithelium of the asthmatics compared to normal control subjects, 2.64% vs 0.445% (p=0.02). No differences were observed in the expression of AGE by inflammatory cells (2.77 vs 1.09 cells/mm2) or in the ECM (0.31 vs 0.74%). This expression of AGE exhibited a negative relationship with FEV1 (rho=-0.48, p=0.06). We have also investigated AGE expression in the bronchial and parenchyma tissue of subjects with COPD and compared it to those without. In the bronchial tissue there was no difference in expression in the epithelium (16.5 vs 10%) or the AGE immunoreactive inflammatory cells (14.1 vs 21.5 cells/mm2) in COPD compared to non- COPD. We also did not observe any disease related difference in the AGE expression in the lung parenchyma (34.9 vs 28.9%). In conclusion, AGE is expression is increased asthma where it appears to be related to lung function, but no increase is observed in COPD. This suggests that there are differences in AGE expression in relation to diseases with variable airflow obstruction versus those with fixed airway obstruction. Further work is needed to investigate the mechanisms by which this may occur and the pathological consequences.

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Modulation of the IgE response by blocking membrane-IgE

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Transgenic mouse experiments in our lab clearly showed that the transmembrane domain of mIgE is indispensable for T-cell dependent IgE secretion and that the cytoplasmic domain not only determines the absolute amount of IgE produced, but also influences the quality of the immunoglobulins. Thus, if mIgE is the prerequisite for the later production of secreted IgE, targeting mIgE bearing B cells with anti-mIgE specific antibodies could be a promising systemic therapeutic approach.

Since IgE antibodies play a key role in allergic disorders, a number of approaches to inhibit IgE antibody production are currently being explored. Treatment with 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 this reduction remains speculative and has to be evaluated carefully. A possible explanation for the reduction of serum IgE may be that these antibodies can also interact with membrane bound IgE on B cells, leading to a clearance of the mIgE population.

In order to investigate the biological mechanism behind an anti-IgE therapy, we used the extra membrane proximal domain (EMPD) of mIgE, as target sequence for generating anti mIgE antibodies with the capacity to inhibit IgE synthesis in the murine system. So far, we were able to isolate two specific single chain antibodies and one specific anti EMPD-monoclonal antibody, showing high specificity for the EMPD-region in ELISA and flow cytometry. Immunization experiments with recombinant Bet v 1 and anti-EMPD in parallel showed a reduction of specific IgE antibodies by more than 90%.

Our hypothesis suggests that cross linking mIgE receptors by anti-EMPD antibodies without the appropriate T cell help leads to direct clonal deletion and / or clonal anergy of the total mIgE B cell population.

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Sublingual Immunotherapy Reduces Allergic Symptoms in a Mouse Model of Rhinitis

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In the present study we have investigated the effect and mechanisms of sublingual immunotherapy (SLIT) in a mouse model of rhinitis.

Mice were sensitized by intraperitoneal (ip) injections of *Phleum pratense* (Phl p) extract adsorbed to alum, followed by sublingual treatment with Phl p-extract or buffer for 6-9 weeks. The mice were subsequently challenged intranasally for two weeks with Phl p-extract and analyzed for clinical symptoms, antibody levels, T-cell proliferation and eosinophilia.

Our results show that ip-sensitized mice that were subsequently challenged intranasally developed rhinitis characterized by increased airway hyperreactivity, increased levels of IgE in serum, washes of the lungs (BAL) as well as nasal passages (NAL), increased influx of eosinophils into nose and lungs and increased inflammation in nasal tissue. In the present study we demonstrate that SLIT treatment is able to reduce the allergic symptoms in the above described model of rhinitis, as sensitized mice, that were SLIT-treated using a dose of 125 kSQ for nine weeks prior to intranasal challenge displayed reduced airway hyperreactivity, reduced levels of Phl p-specific IgE and IgG1 in serum, IgE in BAL and NAL as well as reduced eosinophilia in lungs and nose. Furthermore, T-cell proliferation was reduced in the draining lymph nodes. When mice were treated with a lower dose (25 kSQ) for a shorter period (six weeks), they displayed a minor, non-significant reduction of IgE and IgG1 in serum and no change was observed in clinical symptoms or eosinophilia, indicating that the effect of SLIT is time- and dose-dependent.

In conclusion, our results demonstrate that SLIT-treatment is able to reduce allergic symptoms in a murine rhinitis-model. Furthermore, the effects of SLIT appears to be time- and dose-dependent.

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Anaphylactic and anaphylactoid reactions to paclitaxel, carboplatin and doxorubicin : treatment with rapid desensitization.

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An acute need for rapid desensitizations to chemotherapy drugs has been apparent since more cancer survivors have been exposed to many chemotherapy cycles. Of the patients treated with carboplatin 27 % develop IgE-mediated anaphylactic reactions after 6-8 cycles, preventing the use of an important drug, active against their cancer. Although desensitizations remain empirical and pose high risk by reintroducing a potentially lethal medication into the allergic patient, in

vitro data indicates that suboptimal antigen down regulates syk preventing mast cell and basophil activation at optimal doses, and that STAT6 is implicated.

We have previously standardized a protocol for rapid desensitization to chemotherapy, and used it in patients who had presented IgE-mediated hypersensitivity reactions. Sub-optimal doses were infused for 4 to 6 hours until reaching the optimal dose, without the induction of anaphylaxis.

Objectives: We wanted to provide evidence of efficacy and safety of a 3 solutions, 12 steps desensitization protocol for the treatment of IgE and non-IgE mediated hypersensitivity reactions to first line chemotherapy drugs. We also wanted to demonstrate the feasibility of chemotherapy desensitizations in the out-patient setting.

Methods: Patients with ovarian, breast and other gynecological malignancies and anaphylactic/anaphylactoid reactions to paclitaxel, carboplatin and doxorubicin were evaluated for desensitization. Skin testing was done for carboplatin reactions. Desensitization was performed, starting at 1/100 the optimal dose and by incremental administration of doubling sub-optimal doses at 15 min intervals, until reaching the final dose. The first desensitization was done in the MICU and subsequent desensitizations in the out-patient oncology center.

Results: A total of 110 desensitizations were done in 28 patients : 11 patients received 60 paclitaxel courses, 15 patients received 48 carboplatin courses and 2 patients received 3 doxorubicin courses. All patients receiving carboplatin had a positive skin test. Mild side effects occurred in 3 patients for a total of 3 desensitizations, but no anaphylaxis or anaphylactoid reactions.

Conclusions: Patients with anaphylactic and anaphylactoid reactions to chemotherapy drugs can be safely treated by rapid desensitization using this 3 solutions, 12 step protocol in the out-patient setting, once the safety of the procedure has been established in the in-patient setting.

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Intramuscular immunization with DNA construct containing Der p 2 and signal peptide sequences primed strong IgE production

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Background: Previous studies demonstrated that allergen gene vaccination induced TH1-skewed responses and inhibited IgE production.

Objective: This study evaluated and characterized the immune responses induced by three DNA constructs for vaccination against mite allergy.

Methods: Mice were immunized intramuscularly with DNA constructs encoding a major mite allergen, Der p 2, without a signal peptide (p2), with signal peptide (p52), and with signal peptide plus lysosomal-targeting sequence (p52-LA), respectively, followed by protein boost. Antibody and T-cell cytokine responses were assessed by ELISA. Circulating Der p 2 protein was detected by sandwich ELISA. Primed dendritic cells (DCs) were adoptively transferred to naïve mice, humoral responses were examined after protein challenged.

Results: Mice immunized with p52-LA showed strong and clear-cut TH1-type response, as evident by high IFN-^{3}: IL-4 ratio and elevated levels of Der p 2-specific IgG2a production whereas construct p2 induced only low levels of TH1 response. In contrast, mice immunized with construct p52 showed a mixed TH1/ TH2 phenotype and produced substantial circulating Der p 2 protein. Mice adoptively transferred with DCs primed by p52 but not the p2 or p52-LA were sensitized to produce Der p 2-specific IgE.

Conclusion: DNA construct with signal peptide could prime the IgE production and additional inclusion of lysosomal-targeting sequences reduced the risk of TH2 sensitization.

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Time course analysis of clinical and immunological markers of tolerance during grass pollen immunotherapy

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Rationale: Mechanisms associated with grass pollen immunotherapy include the induction of IL-10 producing 'regulatory' cells and the induction of inhibitory IgG antibodies. This study aimed to establish the time-course of clinical, cellular and humoral responses during a double-blind placebo controlled trial of grass pollen immunotherapy.

Methods: Blood was obtained before and two weekly during a 6-8 week updosing 'cluster' regimen of grass pollen immunotherapy (Alutard SQ) and thereafter monthly/quarterly during 12 months maintenance injections. Serum allergen-specific (*Phleum pratense*) IgG4 was measured by ELISA. Flow cytometry was used to detect serum inhibitory activity for IgE-facilitated CD23-dependent allergen binding to B-cells. Allergen-driven IL-10 was assessed by ELISA of PBMC culture supernatants at 6 days. Early and late phase skin responses at 8 and 24 hours after intra-dermal allergen (10BU, *Phleum pratense*) provided a clinical readout of tolerance induction.

Results: Comparing active and placebo-treated groups, we observed overall significant increases in IL-10 production (p=0.01), decreases early and late phase skin responses (p=0.04, p=0.02), increases in serum IgG4 (p=0.009) and inhibitory activity for IgE-facilitated binding of allergen-IgE complexes to B cells (p=0.004). Analysis of the time course of changes within immunotherapy-treated patients revealed that reductions in late-phase skin responses occurred within 2 weeks (p=0.009) and were associated with corresponding increases in IL-10 production from allergen-stimulated PBMC (p=0.001). These changes occurred at low allergen doses (approximately 3,300 SQ, compared to monthly maintenance 100,000 SQ injections). Increases in IgG4 (p=0.01) and in IgG serum inhibitory activity (p=0.001) occurred later, at 4-6 weeks after commencing treatment. Early phase skin responses were also modestly decreased, but only after 8 weeks therapy (p=0.009).

Conclusion: Following grass immunotherapy the regulatory cytokine IL-10 is produced early (2 weeks) and corresponds to a decrease in late skin responses whereas increases in allergen-specific IgG4 and associated serum 'blocking' activity occur later (4-6 weeks) and are associated with inhibition of IgE-mediated early skin responses.

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Directed molecular evolution of mite group 2 allergen genes generating hypoallergens for allergen-specific immunotherapy

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Allergen-specific immunotherapy (ASIT) is the only treatment in use that gives long-lasting relief of allergic symptoms. Currently, it is based on repeated administration of allergen extracts. Although successful clinical outcome has been documented several problems are associated with allergen extract-based ASIT, e.g., the risk of inducing local or systemic side effects. To improve the safety and efficacy of ASIT the concept of hypoallergenic vaccines, i.e., recombinant allergens with reduced IgE-binding capacity but retained T-cell reactivity, has been suggested. Hypoallergens can be created by molecular modification of recombinant allergens but it may be difficult to predict how to modify a specific allergen to achieve the desired hypoallergenic properties. Directed molecular evolution by DNA shuffling and screening provides a means to evolve proteins having novel or improved functional properties of interest without prior knowledge of the structure-function relationships of the target molecules. In this study we employed multi-gene DNA shuffling using three group 2 mite allergen genes, two isoforms of the major allergen Lep d2 from the dust mite *Lepidoglyphus destructor*, and Gly d2 from the related mite *Glycophagus domesticus*, with the aim of creating hypoallergens. DNA shuffling yielded a library of shuffled genes from which encoded shuffled allergens were expressed and tested in two consecutive screenings. A positive selection was made for full-length expressed clones with an intact C-terminal epitope. Shuffled allergens were negatively selected for low binding to IgE from a pool of sera from nine mite allergic patients using an IgE bead-based binding assay. The IgE-binding assay was then used in competition experiments with wild-type Lep d2 to characterize the low-IgE binding shuffled allergens. Nine shuffled allergens, with IgE-binding ranging from approximately 80-fold reduced to completely abolished compared to the parental allergens, were chosen for further analysis of antigen-specific T-cell proliferation. Two of the shuffled allergens stimulated T-cell proliferation equally well as the wild-type allergens in peripheral blood mononuclear cell cultures originating from seven *L. destructor* sensitised patients. In conclusion, we here show that directed molecular evolution is a powerful method to generate allergens with hypoallergenic properties for potential use in ASIT.

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Adsorption of Allergens to Aluminium Hydroxide Adjuvant Induces Only Minor or No Structural Changes of the Allergen

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Background: Aluminium hydroxide has been used as adjuvant in allergy vaccines for decades. The immunological responses obtained probably depend on the physico-chemical state of the adsorbed allergens. It has been very difficult to gauge the structural integrity of the adsorbed allergens, thus little is known about the physical state of the adsorbed allergens. Here we show that fluorescence spectroscopy can be used to probe the folding state of a purified single allergen in a formulated vaccine.

Experimental Methods: The folded state of adsorbed nPhl p 1 and rDer p 2 to aluminium hydroxide was monitored by fluorescence spectroscopy. Changes in the environment of tryptophane residues (hydrophobic to hydrophilic) were monitored as the shift in the maximum emission wavelength. The degree of unfolding was estimated relative to the spectral changes observed from a chemically induced denaturation of the allergen in solution.

Results: The adsorption of rDer p 2 to aluminium hydroxide did not induce remarkable shifts in the emission maximum, indicating that rDer p 2 maintains its native fold after adsorption. The adsorption of nPhl p 1 seem to perturb the folded state to a minor degree, since the observed shift in emission maximum corresponds to 80% of the molecules maintaining their native fold after adsorption to aluminum hydroxide.

Conclusion: The adsorption of purified single allergens to aluminium hydroxide affects the folded state of the adsorbed allergen to a minor extent, indicating that the active ingredients in aluminium hydroxide vaccines can maintain their native fold after formulation.

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Development of a mucosal polyvalent allergy vaccine for primary prevention of multi-sensitization

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Based on clinical data showing that many allergic patients become co-sensitized to several allergens and these patients are very difficult to treat by conventional immunotherapy, our research has focused on the development of novel treatment strategies based on mucosal tolerance induction for poly-sensitized patients.

We therefore established a mouse model of polysensitization to the major birch and grass pollen allergens Bet v 1, Phl p 1 and Phl p 5, to evaluate if mucosal tolerance can be simultaneously induced to several allergens. In contrast to a mixture of the complete allergens, mucosal pre-treatment with a mixture of the immunodominant peptides or a hybrid-peptide prevented allergic poly-sensitization to all three aeroallergens. The underlying mechanisms of poly-peptide induced tolerance seemed to be regulated by anergy rather than regulatory cytokines.

As we demonstrated that the native conformation of an allergen is of importance for maintenance of tolerance, we sought to improve poly-tolerance induction by creating an allergen chimer, consisting of a so called host allergen in its native conformation anchoring the immunodominant peptides of two or more non cross-reactive guest allergens. Bet v 1, being a strong tolerogen for induction of long term immunosuppression via regulatory mechanisms, served as a scaffold for linkage of the immunodominant T cell epitopes of Phlp 1 and Phlp 5. Indeed, intranasal tolerance induction with this chimer led to suppressed allergic responses to all three allergens, mediated by regulatory mechanisms.

This prototype allergen-chimer now provides the basis for further construction of chimers against seasonal and perennial allergies as well as pollen associated food allergy.

Following the concept of polyvalent paediatric vaccines to prevent most dangerous infectious diseases, prophylactic application of a mucosal polyvalent allergy vaccine, covering the most common allergens, could be a future strategy of early allergy prevention in atopic individuals and children at risk.

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IgE-Regulatory Effects of Histamine in Atopy and Non-Atopic Diseases

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Histamine plays a crucial role in the pathogenesis of IgE-mediated allergic as well as non-allergic diseases. The inflammatory role of histamine and involvement of different types of histamine receptors is well established. On the same time histamine via its specific receptors may modulate an IgE synthesis and this effect is less investigated. Our data showed that IgE regulation induced by histamine is mediated via H_1 and H_2 as well as H_3/H_4 receptors. It has been shown that histamine involves in IgE response not only in allergic diseases (allergic rhinitis, asthma, atopic dermatitis) as well as in non-allergic diseases, such as malignant bone marrow alterations. Histamine-mediated effects on IgE synthesis can be realized in these diseases via not only well-investigated H_1 and H_2 receptors, as well as via newly discovered and recently cloned H_3 and possibly H_4 receptors (?). IgE synthesis induced by histamine is highly dependent on the concentration of mediator and type of involved receptors. Thus, histamine in high concentrations (10^{-5}M) suppressed and in low concentrations (10^{-8}M) stimulated spontaneous IgE synthesis. Antagonists of H_1 (loratadine) and H_2 (cymetidine) receptors oppositely influence on IgE response in MNC cultures of ragweed sensitive patients and patients with lympho- and erythroproliferative diseases. The study of several imidazole and non-imidazole H_3/H_4 antagonists (FUB 181, FUB 649, FUB 372, Imoproxifan-IMP and Ciproxifan -CF) showed that IgE synthesis highly depends on the potency and structural differences of investigated H_3/H_4 antagonists. IgE regulation induced by histamine is closely related to well-known IgE-regulatory cytokine mechanisms. IgE synthesis modulated by histamine and several histamine receptor antagonists (H_1, H_2 and $H_{3/4}$) is highly dependent on the IL4, as well as γIFH and IL13 production. Underlying mechanisms of IgE regulation modulated by histamine and its antagonists may be different in atopy and bone marrow alterations.

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Antibody responses to minor allergen Bet v 2 during allergen specific immunotherapy in birch pollen allergic patients

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Background: Allergen specific immunotherapy (SIT) induces small changes in the level of specific IgE but most pronounced is a quantitative increase in allergen specific IgG. The effect on antibodies to minor as compared to major allergens is not clear. In this study antibodies towards Bet v 1 and Bet v 2 of *Betula verrucosa* were investigated in sera of SIT treated patients.

Methods: Specific antibodies were assayed by the binding of ^{125}I labelled purified natural Bet v 2 and recombinant Bet v 1.2801. Specific IgE was assayed following binding to anti-IgE monoclonal antibody coupled to paramagnetic beads. IgE depleted serum was tested for specific IgG and following incubation with the labelled allergen, free and bound allergen were separated by protein G affinity chromatography. Sera were tested in immunoblotting with or without absorption using 1 mg birch pollen extract.

Results: All sera (n=50) were positive for IgG and IgE against Bet v 1 before and after SIT. The relative binding activities of IgG compared to IgE were up to 30 times higher after SIT. In contrast, 22% of non-treated patients were positive for IgE anti-Bet v 2 and 48% for IgG anti-Bet v 2. At 1.5 years of SIT, the prevalence of IgG- and IgE-anti-Bet v 2 was unchanged, however, after 5 years of treatment, the prevalence of IgG anti-Bet v 2 was 91% but the prevalence of IgE anti-Bet v 2 was unchanged when analysed by quantitative immunoassay. Contrary to this observation, 3 sera became IgE anti Bet v 2 positive after 5 years of treatment when measured by immunoblotting.

Conclusion: The dominant binding activity against Bet v 1 and Bet v 2 in sera from birch pollen allergic patients both before and after SIT resides in the IgG fraction. Prolonged SIT treatment induced IgG against minor allergens in most individuals. New IgE reactivities to Bet v 2 were observed after SIT in 3 patients by immunoblotting, however, this result was not confirmed by quantitative immunoassay and may possibly indicate differences in assay sensitivity with respect to denaturation insensitive epitopes.

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Alpha Melanocyte Stimulating Hormone and Fragments: Potential Therapeutic Agents in Inflammation

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α-Melanocyte stimulating hormone (α-MSH) exerts numerous immunomodulatory and anti-inflammatory activities, which at least partly are mediated through the melanocortin receptor 1 (MC-1R), expressed on monocytes, dendritic (DC)-, endothelial-, mast-, and epithelial cells. Accordingly, α-MSH downregulates the production of proinflammatory cytokines and the expression of costimulatory molecules on antigen presenting cells via inhibiting the activation of transcription factors such as NF-ºB, while upregulating the production of suppressor factors such as IL-10. Besides α-MSH its C-terminal-tripeptide (KPV) and the IL-1² derived tripeptide KPT are capable of modulating APC functions. Using a mouse model of contact hypersensitivity (CHS) systemic and epicutaneous application of α-MSH, KPV or KPT inhibited CHS induction and induced hapten-specific tolerance. However, using MC-1R deficient mice (MC-1Re/e) tolerance induction was found to be independent of MC-1R expression. To further investigate the mechanisms responsible for tolerance induction adoptive transfer experiments were performed. Accordingly, α-MSH treated haptenized DC inhibited CHS and induced hapten-specific tolerance, via the induction of regulatory T-lymphocytes (T^{reg}). In contrast, using a murine model of intestinal inflammation (Dextransulfate (DSS) induced colitis) the expression of a functional MC-1R was found to be crucial for α-MSH to exert its anti-inflammatory activity. In *wt* mice weight loss was reduced and the survival rate was significantly improved upon treatment with α-MSH or KPV. However, DSS colitis was significantly aggravated in MC1-Re/e mice, resulting in death of all animals. Bone marrow transplantion from *wt* mice did not alter the course of inflammation, indicating that MC-1R expression on non-hematopoietic cells is crucial for host defense. These findings further support the therapeutic potential of α-MSH related peptides for the treatment of inflammatory, autoimmune and allergic diseases.

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Discovery of LAS 36674, a new generation of H_1 antihistamines: from bench to bedside and back

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H_1 antihistamines are still the first-line medication for patients with allergic rhinitis. They prevent symptoms associated with histamine release such as sneezing, rhinorrhea, nasal and conjunctival itching and lachrymation, although they do not control nasal congestion. The second-generation of H_1-receptor antagonists have a greatly improved benefit/risk ratio compared with the first-generation antihistamines, in term of their reduced potential to cross the blood-brain barrier and their higher receptor specificity. Unfortunately, life-threatening adverse cardiac effects such as QT interval prolongation and *torsades de pointes* (specific polymorphic ventricular tachyarrhythmia) have been associated with the use of some second-generation antihistamines such as terfenadine and astemizole. Indeed, several second-generation H1-antihistamines interact with CYP450, in particular the subtype CYP3A4, which, in the case of compounds with inherent risk of side-effects, may cause potentiation when polymedication occurs. The objective of our work was to develop potent, selective and long lasting H1 antihistamines devoid of cardiotoxicity, sedative effects and drug-drug interactions that may be favourably differentiated with respect to the second-generation antihistamines. LAS 32928, a indolylpiperidinyl benzoic acid derivative with a favourable preclinical profile, was initially selected for clinical development. In healthy adult volunteers both single and multiple oral dose regimes of up to 50 mg and 25 mg, respectively, were very well tolerated. The maximum mean percentage reduction in cutaneous histamine-induced wheal area was 78 and 93 % after single dose administration of 5 and 10 mg, respectively. The pharmacokinetic profile showed an early Cmax and a short elimination half-life. In order to improve the duration of action and pharmacokinetic profile of LAS 32928 new indolylpiperidinyl derivatives were synthesized. LAS 36674 was selected as a candidate for development based on its preclinical pharmacological and ADME profile: potent and long lasting antihistamine activity in rats, low brain penetration in mice and rats, lack of cardiotoxicity (no effects on K^+ hERG channel, APD-90 and QTc interval), no significant interaction with CYP450 isoforms and long elimination half-life in rats. Overall, LAS 36674 demonstrates a superior efficacy / safety ratio than second-generation antihistamines in preclinical models, suggesting the potential for a superior benefit/risk profile in humans.

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A potent adenosine A2B receptor antagonist attenuates methacholine-induced bronchial hyperresponsiveness, mucus production and IgE levels in an allergic mouse model

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Substantial experimental evidence highlights the importance of adenosine in the pathogenesis of asthma and COPD. Inhaled adenosine causes dose-related bronchoconstriction in patients with asthma and COPD but not in healthy volunteers, and this response appears to be orchestrated mainly by adenosine-activation of mast cells through one of the four described adenosine receptors (A1, A2A, A2B, A3). Recently it has been shown that an adenosine A2B receptor antagonist (CVT-6883) prevents AMP-induced bronchoconstriction in an allergic mouse model (ATS 2005, Fan et al., poster H75) and attenuates pulmonary inflammation, fibrosis and alveolar airway enlargement in adenosine deaminase (ADA)-deficient mice (ATS 2005, Sun et al., poster C34). These results suggest that the A2B receptor may play a role in chronic lung diseases. The objective of our work was to study the effect of LAS 38096, a potent (Ki of 46 nM for the human receptor) and selective adenosine A2B receptor antagonist, on methacholine-induced bronchoconstriction, lung inflammation, mucus production and IgE levels in an allergic mouse model. Female Balb/c mice were sensitised with two injections (i.p.) of ovalbumin (OVA, 10 µg) plus Alum (2mg/ml) on days 1 and 10. Aerosol challenges were performed with 5% OVA for 20 min during 6 consecutive days from day 19 to 24. LAS 38096 was administered orally twice daily 1 hour before- and 6 hours after- each OVA challenge. 24 hours after last OVA-challenge, methacholine-induced bronchial hyperresponsiveness (BHR) was measured using whole body plethysmography (Buxco). A bronchoalveolar lavage (BAL) was then performed for cellular infiltration determination, and lung tissue and blood samples were collected for histopathology and OVA-specific IgE level measurements, respectively. Compared to PBS-challenged mice, untreated OVA–challenged mice showed a significant increase in BHR, eosinophil lung infiltration, BAL Th2 cytokines and plasma OVA-IgE levels, and airway mucus production. In contrast, OVA-challenged mice treated with LAS 38096 showed significantly less BHR, mucus production and OVA-specific IgE levels and a slight decrease in eosinophil infiltration and Th2 cytokine levels. Overall, these results suggest that blockade of the A2B receptor may provide clinical benefits in the treatment of chronic respiratory diseases.

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Vaccination with genetically modified birch pollen allergens has beneficial effects on birch pollen allergy-associated oral allergy syndrome

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Background: Patients suffering from Type I allergy to birch pollen also develop oral allergy syndrome (OAS). OAS is caused by cross-reactivity between the major allergen from birch (Bet v 1) with the major allergens from apple (Mal d 1), celery (Api g 1), carrot (Dau c 1), and other plant food allergens. We studied the effects of immunotherapy with genetically modified derivatives of Bet v 1 on food allergy symptoms in patients with OAS.

Methods: Bet v 1-derivatives (Bet v 1-trimer, Bet v 1-fragments) were used for the treatment of birch pollen allergic patients in a double-blind, placebo-controlled study. The effect of this new treatment on OAS symptoms was investigated in a sub-study. Symptoms caused by food ingestion were assessed using questionnaires before and after therapy. IgG_{1-4} antibodies against Mal d 1, Api g 1 and Dau c 1 induced by immunotherapy were measured by ELISA. Whether treatment-induced Bet v 1-specific IgG antibodies inhibit food allergen induced effector cell activation was studied in basophil histamine release experiments.

Results: After one pre-seasonal treatment course OAS symptoms had improved in 7/23 patients in the treatment group and in 1/19 patients in the placebo group. Symptoms had deteriorated in 2/23 actively treated and in 2/19 placebo-treated patients, and had remained unchanged in the other patients. Immunotherapy with Bet v 1 derivatives induced IgG antibodies to Mal d 1, Api g 1 and Dau c 1. Histamine release

experiments revealed that these IgG antibodies reduced food allergen induced basophil degranulation and were thus of protective nature.

Conclusion: Immunotherapy with Bet v 1-trimer and Bet v 1-fragments induces blocking IgG antibodies against Bet v 1-cross reactive food allergens and may improve symptoms of food allergy in patients with Bet v 1-associated food allergy.

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Clinical improvement and immunological changes of Atopic Dermatitis in patients undergoing subcutaneous immunotherapy with depigmented polymerized house dust mite allergens

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Purpose: House dust mites (HDM) such as *Dermatophagoides pteronyssinus (Der p)* and *Dermatophagoides farinae (Der f)* are the most important indoor allergen sources for patients with atopic dermatitis (AD). Since allergen-reduction achieved by encasing strategies does not lead to significant improvement of the clinical symptoms, subcutaneous immunotherapy (SIT) against HDM might represent an attractive therapeutic option for the long-time treatment of these patients. However, studies on the effectiveness of HDM SIT in patients with AD have provided controversial clinical results. Furthermore data on the immunological changes induced by SIT in AD patients are rare. Therefore HDM SIT is currently not accepted as standard therapy for AD. To evaluate the therapeutic value and immunologic response of patients with AD under SIT, we performed HDM SIT with a depigmented polymerised HDM extract in a total of 24 adult AD patients (mean age 31.9±18.2 years) with moderate to severe AD (mean subjective SCORAD 44.31, mean objective SCORAD 36.52) and sensitizations against *Der p* and *Der f* evaluated by positive CAP RAST >3, positive Prick Test and/or positive Atopy Patch Test to *Der p* and *Der f*.

Results: Subjective and objective SCORAD improved statistically significant already within 4 weeks of treatment in >80% of the patients (p=0.001). A statistically and clinically significant reduction of the objective SCORAD of 48.6% (p<0.001) and the subjective SCORAD of 52% (p<0.001) was obtained after 6 months of treatment. In patients with a positive atopy patch test this effect was even more pronounced. The concomitant treatment predominately consisted of emollients, topical immunomodulators and corticosteroids was reduced by 6-7% each after 6 months of treatment. Generally SIT was well tolerated. 2 patients withdrew related to side effects: 1 patient due to worsening of AD and 1 patient due to bronchial obstruction. Further on, suppressive activity of regulatory T-cell subtypes, the level of the tolerogenic cytokines IL-10 and TGF-β combined with the amount of chemokines correlating with the disease severity such as TARC/CCL17 in the sera of the patients changed during SIT.

Conclusion: SIT with a depigmented polymerised HDM extract leads to significant improvement of the subjective and objective SCORAD as well as immunological markers known to go along with the therapeutical effect of SIT in patients suffering from moderate to severe AD sensitised against HDM.

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Induction of allergen-specific CD8$^+$ T cells with protective effects against allergic airway inflammation

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Studies in humans and rodents have indicated that in addition to CD4$^+$ T cells, CD8$^+$ T cells are also important in mediating and regulating allergic inflammation. In this study, we used major histocompatibility complex (MHC) class I multimer technology (tetramers) to analyze the induction, the natural distribution, the phenotype and the contribution of allergen-specific CD8+ T cells in a murine C57BL/6 model of alum-ovalbumin (OVA)-induced IgE-mediated allergy, a model that was typically characterized by the induction of OVA-specific IgE and IgG1 together with airway eosinophilia following OVA aerosol exposure. Using the $H2_{kb}$ tetramer complexed with the immunodominant OVA peptide SIINFEKL ($OVA_{257-264}$) we observed up to 40% $OVA_{257-264}$-specific CD8+ T cells, both in lung tissue and in bronchoalveolar lavage (BAL) fluid of sensitized mice after repetitive OVA aerosol challenge. The majority (95-100%) of CD8$^+$/tetramer$^+$ cells showed an effector phenotype (CD62L$^-$, CD69$^+$, CD127$^-$, CD44$^+$, CD25$^{+/-}$) and strong IFN-γ but no IL-4 production, characteristic of a Tc1 pattern. Depletion of CD8$^+$ T cells by monoclonal antibody prior to OVA aerosol challenge strongly enhanced the resulting airway eosinophilia. Adoptive transfer experiments with $OVA_{257-264}$-specific CD8$^+$ T cells from transgenic OT-1 mice showed migration of naïve allergen-specifc CD8$^+$ T cells to the lungs of wild-type recipients and specific proliferation in lung tissue with a Tc1 pattern after OVA aerosol challenge. However, no reduction in airway eosinophilia was achieved by transferring CD8$^+$ T cells from OT-1 mice. In contrast, the adoptive transfer of antigen-primed $OVA_{257-264}$-specific CD8$^+$ T cells prior to airway allergen challenge showed a variable degree of reduction of airway eosinophilia, dependent on the context of T cell priming. Lengthy time delay between allergic sensitization and allergen challenge, conditions of antigenic overload, which resemble the situation in specific immunotherapy, and antigen presentation in the context of bacterial signals are favourable factors for inducing a protective $OVA_{257-264}$-specific CD8$^+$ T cell response. Thus, our data suggest defined conditions for a protective role of allergen-specific CD8$^+$ T cells in the regulation of airway inflammation, which may be due to their specific cytokine and migration pattern, and most importantly to the context of antigen priming. (support: BMBF grant 01GC0104)

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Hymenoptera Venom Allergy: A New Ultra-Rush Immunotherapy

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Introduction: Different frequencies of side effects have been reported during venom immunotherapy (VIT) depending on different protocols and venom preparation quality. The aim of the study was to assess the tolerability of a new ultrarush protocol compared to rush and conventional protocols and to investigate the safety aspects when updosing treatment with aqueous preparation was switched to aluminium hydroxide adsorbed extract in maintenance phase.

Methods: Seventy-five patients (51 M, 24 F; aged 16-76 years) with history of systemic anaphylactic reactions to insect venom were treated according to three different regimens. All patients had been treated with standardized, purified venom preparations (Alk-Abello'). During updosing phase, patients in group A (Vespidae:18; Apidae:9; n=27) or group B (Vespidae:16; Apidae:9; n=25) received an aqueous preparation with ultra-rush protocol in 195 minutes and with rush protocol in three days, respectively. Patients in group C (Vespidae:16; Apidae:7; n=23) were treated with conventional VIT using an aluminium-hydroxide adsorbed preparation. Maintenance dose (100 microg) was administered with adsorbed preparation after 15 days and thereafter once a month in all groups.

Results: Treatment with ultrarush protocol evoked large local reactions less frequently than rush and conventional protocol in updosing phase [group A:7/351 (1,9%); group B:12/375 (3,2%); group C:12/110 (10,9%) P<0.05] whereas no difference was observed in maintenance therapy. During dose increase phase no systemic side-effects were observed in patients with ultrarush VIT compared to other protocols [group A: 0/351; group B: 2/375 (0.6%); group C: 1/110 (0.9%)]. No patient who has stung accidentally by a hymenoptera had a large local or systemic reaction.

Conclusions: These data show efficacy of VIT with rapid protocol that conferred permanent protection similarly to other protocols. In addition, ultra-rush– protocol was safer and better tolerated as likely the quality of life is more care in ultra-rush protocol than other protocols. Our observations suggest the possibility to shift from an aqueous preparation used for incremental phase to an adsorbed preparation used for maintenance phase.

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A follow-up study of immunotherapy-treated birch-allergic patients. The effect on expression of chemokines in nasal biopsies.

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SIT	specific immunotherapy
RANTES	regulated upon activation normal T-expressed and secreted (CCL5)
TARC	thymus and activation-regulated chemokine (CCL17)
DBPC	double blind placebo controlled

Background: Specific immunotherapy (SIT) is the only treatment that offers long lasting clinical improvement. Chemokines are essential for the migration of inflammatory cells to the target tissue during allergic reaction. We investigated the long term effect of SIT treatment on the expression of eotaxin, RANTES and TARC in biopsies of nasal mucosa.

Methods: Sixteen patients who finished 3-5 years ago 3-years treatment with birch SIT were recruited from an DBPC trial to participate in follow-up study. Twelve matched birch-allergic patients were included as a control group. The inclusion/exclusion criteria were the same for control group as was the original SIT study. All patients recorded rhinoconjunctivitis symptoms and use of rescue medication before and during the pollen season. Nasal mucosa samples were obtained before and around the peak of the pollen season. The biopsies sections were stained using markers for eosinophils (eosinophil peroxidase), mast cells (anti-tryptase) and antibodies against chemokines: eotaxin, RANTES and TARC using immunocytochemical methods.

Results: During the season rhinoconjunctivitis symptoms increased in both SIT and control groups (p=0.001 and p=0.002, respectively). However, SIT patients had 37% less (p=0.01) symptoms than controls. Also, the medication score increased in both groups (p=0.002) during the season but the SIT group used 28% less medication than the control group (p=0.02). The number of eosinophils increased in the controls (p=0.01) and the difference between groups was significant during the season (p=0.01). No seasonal increase in numbers of mast cells was found but there was more (p=0.02) mast cells in control than in SIT group during the season. The number of eotaxin$^+$ and RANTES$^+$ cells increased only in the control group (p=0.01 and p=0.03, respectively) and the difference between groups was significant during the season (p=0.01; p=0.01 respectively). Number of TARC$^+$ cells was lower in SIT treated patients during the season (p=0.003), however we haven't found seasonal increase in neither of groups.

Conclusion: This study confirmed the long-lasting effect of birch SIT which is accompanied by decreased numbers of eosinophils in the nasal mucosa. SIT prevented also seasonal rises in the number of cells expressing eotaxin and RANTES.

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Pharmacodynamics of latest generation H_1 antihistamines: relevance of drug concentrations at receptor sites and of affinity values for H_1 receptors

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Introduction: The percentage of receptor occupancy (RO) has been shown to correlate with the percentage of inhibition of histamine-induced wheal and flare by an H_1-antihistamine (Gillard et al., Inflammation Research 2005). The estimation of RO requires the availability of both the drug concentration at receptor sites (RS) and affinity of the drug for the receptor. Classically, free plasma concentrations, calculated from plasma concentrations using plasma protein binding, are used as an approximation of the drug concentration at RS. The purpose of this work is to estimate the drug concentration at RS by an alternative approach, using the volume of distribution and the skin concentrations of the drug.

Methods and Results: Skin concentrations were measured by validated methods in samples from 18 adult allergic volunteers 24 h after administration of 5 mg oral levocetirizine or desloratadine. The mean values were 48.9 and 69.1 ng/g, respectively **(n = 13).** The volumes of distribution (V/F) of levocetirizine and desloratadine are 28 and 3430 L, respectively for a 70 kg man. Using the expression

$$V/F = V_P + \frac{f_{uP}}{f_{uT}} \bullet V_{TW}$$ where F = absolute bioavailability (assumed to be 1), V_P = volume of plasma (3.5 L for a 70 kg man), V_{TW} = volume in which the drug is distributed outside plasma (approximated to extracellular water or total body water, 15 or 42 L for a 70 kg man), f_{uP}= fraction unbound in plasma and $f_{uT=}$ fraction unbound in tissue, it is possible to calculate f_{uT} (0.154-0.055 for levocetirizine and 0.0018-0.0007 for desloratadine) and therefore the free tissue (skin) concentration by the relationship $f_{uT} \cdot C_T$, where C_T is the total tissue (skin) concentration. Based on these calculations, free skin concentrations ranged between 2.69-7.53 and 0.048-0.12 ng/g for levocetirizine and desloratadine, respectively.

Conclusion: The estimated free skin concentration values are very similar to free plasma concentrations. Therefore, the free skin/plasma concentrations should be used to estimate the RO by an H_1-antihistamine. High skin concentrations simply reflect an extensive distribution of a drug (skin H" 19% of body volume) and not necessarily the drug concentration at RS, whereas high free skin concentrations are crucial for the efficiency of a drug given for skin allergic problems.

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Development of a Vaccine for the Treatment of Fish Allergy

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Parvalbumin, a small calcium-binding muscle protein, with remarkable resistance to heat and denaturing agents, represents the major and sole allergen for 95% of patients suffering from IgE-mediated fish allergy. Specific immunotherapy, the only curative treatment of type I allergy, is not recommended for fish allergy due to the risk of inducing severe anaphylactic reactions by systemic application of fish allergens.

We produced a folded recombinant carp parvalbumin in *Escherichia coli* which displayed immunological features comparable to natural carp parvalbumin and contained the majority of fish-specific IgE epitopes. In order to develop a vaccine for the safe treatment of fish allergic patients, we introduced point mutations in the two calcium-binding domains of carp parvalbumin by site-directed mutagenesis of the carp parvalbumin encoding cDNA. Three parvalbumin mutants containing amino acid exchanges either in one (single mutants) or in both of the calcium-binding sites (double mutant) were obtained. Of these derivatives the double mutant showed the greatest reduction of overall protein fold and the greatest loss of IgE reactivity. Basophil histamine release assays revealed a profound reduction of the allergenic activity of this mutated parvalbumin. The therapeutic potential of the double mutant was demonstrated by the fact that mouse antibodies raised against the mutated molecule inhibited the binding of fish allergic patients' IgE to the wild-type allergen. We suggest the hypoallergenic carp parvalbumin double mutant as a candidate molecule for immunotherapy of fish allergy.

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Anti-IgE treatment overcomes intolerability of honeybee-venom ultra-rush immunotherapy in indolent systemic mastocytosis

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Immunotherapy with hymenoptera venom reduces the risk of anaphylaxis with subsequent stings and is recommended in patients with systemic reactions and evidence of venom-specific IgE. Patients with elevated basal serum tryptase levels/mastocytosis are at risk for fatalities following stings and often are unable to undergo venom immunotherapy due to intolerable side effects. Otherwise avoidance of hymenoptera stings and use of epinephrine self-injectors might not prevent fatal reactions. Treatment with honey bee venom induces systemic anaphylactic reactions more frequently than yellow jacket venom.

We report the first case of a patient with indolent systemic mastocytosis in whom a single dose of omalizumab enabled ultra-rush honey bee venom immunotherapy within two days. Thereafter, maintenance dose of honey bee venom was tolerated. Tolerability was associated with an early decline of cellular activity assays. In detail, CD63-expression and leukotriene production decreased within one to three weeks, the latter beeing stable for at least 17 weeks. This case demonstrates several important facts: skin testing with honey bee venom remained positive two weeks after omalizumab, basophil CD63 expression to anti-FcμRI± mAb, fMLP, and honey bee venom decreased with a maximum after three to five weeks after the single dose of omalizumab and thereafter increased above original levels. fMLP acts through a distinct receptor that has a markedly different signal transduction pathway from IgE. Cysteinyl leukotriene production to honey bee venom decreased with a maximum two weeks after omalizumab and stayed on this low level for at least 17 weeks.

Taken together this case nicely demonstrates some unsolved problems in the diagnosis and management of hymenoptera venom allergies in patients with elevated tryptase/mastocytosis and shows for the first time that anti-IgE treatment with a single dose of omalizumab enables ultra-rush honey bee venom immunotherapy in risk patients with elevated tryptase levels.

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A hypoallergenic vaccine obtained by tail-to-head restructuring of timothy grass pollen profilin, Phl p 12, for the treatment of cross-sensitization to profiling

Kerstin Westritschnig, Margit Focke, Tea Pavkov, Walter Keller, Tanja Ball, Adriano Mari, Arnulf Hartl, Josef Thalhamer, Fatima Ferreira, Stefan Vieths, Alexandra Böhm, Peter Valent, Rudolf Valenta

Profilins are highly cross-reactive allergens in pollens and plant food. In a paradigmatic approach, the cDNA coding for timothy grass pollen profilin, Phl p 12, which contains the majority of profilin-specific IgE epitopes, was used as a template to develop a new strategy for engineering an allergy vaccine with low IgE reactivity. Non-IgE-reactive fragments of Phl p 12 were identified by synthetic peptide chemistry and restructured as a new molecule, Phl p 12-rs. It comprised the C-terminus of Phl p 12 at its N terminus and the Phl p 12-N terminus at its C terminus. Phl p 12-rs was expressed in E.coli and purified to homogeneity. Determination of secondary structure by circular dichroism indicated that the restructuring process had reduced the IgE-reactive alpha-helical contents of the protein but retained its beta-sheet conformation. Phl p 12-rs exhibited reduced IgE binding capacity and allergenic activity in allergic patients. IgG antibodies induced by immunization of rabbits with Phl p 12-rs cross-reacted with pollen and plant food-derived profilins from various plants and inhibited allergic patients' IgE antibody binding to these profilins to a similar degree as those induced by immunization with the wildtype. Moreover, Phl p 12-rs specific IgG inhibited profilin-induced basophil degranulation. In conclusion, a restructured recombinant vaccine was developed for the treatment of profilin allergic patients. The strategy of tail-to-head reassembly of hypoallergenic allergen fragments within one molecule represents a generally applicable strategy for the generation of allergy vaccines.

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Screening for cellular changes during grass-specific subcutaneous immunotherapy

Peter Adler Würtzen, Anders Millner, Gitte Lund, James Francis, Steven Durham, Kaare Lund

Rationale: Specific Immunotherapy (SIT) is the only known treatment for allergic disease that modulates the immune response causing the disease. The current study was designed to investigate several changes in cellular parameters during SIT treatment previously described in separate studies.

Methods: PBMC from 6 SIT treated and 6 untreated grass allergic patients as well as 8 controls were stimulated directly after isolation or after a subsequent depletion of CD25+. The effect of regulatory cytokines in the cell cultures were investigated by blocking with anti-IL-10 receptor and anti-TGF-beta antibodies. Proliferation, cytokines, intracellular cytokines and surface markers were measured after 6 days of incubation with medium, purified grass allergens, grass extract or tetanus toxoid (TT). Surface markers were also measured on purified PBMC. Histamine release and basophil activation markers were measured on whole blood after stimulation with medium or grass extract for 1 hour.

Results: SIT treated patients produced significantly more IL-10 compared to the other groups when stimulated with grass allergens but not TT. Neutralisation of regulatory cytokines induced a significant increase in allergen-induced IL-5 production only in the SIT group. No changes in proliferation, surface markers or intracellular cytokine levels were observed. All basophil activation parameters are reduced after treatment with SIT compared to the untreated group.

Conclusion: SIT induces a significant increase in IL-10 production and the increase in IL-5 production after neutralisation of regulatory cytokines indicates a modulated T-cell response after immunotherapy. The immediate response from basophils is reduced as a consequence of SIT.

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Allergenicity and Immunogenicity of Allergoid Products commercially available for Birch Pollen Immunotherapy

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Background: The clinical efficacy of specific immunotherapy (SIT) with intact allergens has been documented in a large number of clinical trials. In an attempt to eliminate the small but significant risk of inducing systemic allergic reactions Marsh and co-workers in the seventies proposed the 'allergoid' concept to reduce allergenicity and maintain immunogenicity by chemically modifying the allergen extract. Several commercially available allergoids are currently on the market, however, a statistical evaluation of adverse events reported to the German health authority over a 10 year period did not indicate higher safety of allergoids compared with intact allergens.

Methods: Four commercially available allergoids were compared to Alutard SQ in laboratory assays. Allergenicity was investigated by measuring the inhibition of the interaction between biotinylated Bet v1 and IgE by the different allergen products in solid phase IgE inhibition analyses and by histamine release assays, and immunogenicity by human T-cell proliferation using lines from allergic individuals and measurement of IgG titers following mouse immunizations. The SIT products were normalized with respect to the manufacturers recommended maintenance dose.

Results: Two of 4 allergoids tested did not show reduced IgE binding in the solid phase IgE inhibition analyses compared with the intact allergen product; different slopes of the inhibition curves for the allergoids indicate structural changes of the epitope composition. One of the 4 allergoid products did not show reduced histamine release compared to the intact allergen extract, patient-to-patient variation in histamine release assays was very large. All allergoids showed a reduced response in standard T-cell stimulation assays compared to Alutard SQ® regardless of the cell type used for antigen presentation (PBL or DC). Mice immunized with the allergoid preparations responded with a lower IgG titer to purified rBetv 1 and variation was large whereas all mice immunized with Alutard SQ® showed equally well responses.

Conclusions: Commercial allergoid preparations do not fulfil the allergoid concept, since all allergoids showed reduced immunogenicity, and one allergoid did not show reduced allergenicity. Our results combined with the substantial clinical documentation indicate that treatment with the intact allergen product Alutard SQ® has a preferential risk-benefit-relation compared with chemically modified allergoids.

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Integration of regulatory T cells into the Th1/Th2 paradigm

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Allergy is characterized by allergen sensitization, driving T cell differentiation towards Th2 phenotype. Infectious or autoimmune diseases promote Th1 differentiation excluding Th2 development, which gave rise to the Th1/Th2 paradigm. Accordingly it was hypothesized that allergy origins in an imbalance of Th1 and Th2 cell differentiation. Recent evidence highlighted that regulatory T cells (T_{regs}) control the expansion of effector cells both of the Th1 and Th2 type. However, the origin of the T_{regs} is unclear and therefore also the concepts to integrate T_{regs} into the Th1/h2 paradigm of T cell differentiation.

To understand the origin of T_{regs} we investigated the promoter of the FOXP3 transcription factor, which is decisive for T_{reg} differentiation as T-BET for Th1 and GATA-3 for Th2 cells. Following localization and confirmation of the FOXP3 promoter, using reporter-gene assays, we systematically identified positive regulators of the FOXP3 genes. NFAT, induced by T cell receptor engagement turned out to be essential for FOXP3 induction and explains why FOXP3 is completely inhibited by Cyclosporine A, which was confirmed by mRNA and protein analysis. Of note, glucocorticoids enhance while rapamycin leave FOXP3 expression unchanged, suggesting treatment opportunities with already established immunosuppressants to target T_{regs}. Interestingly we identified GATA-3 and T-BET binding sites between the NFAT and the transcription binding sites. Both sites negatively regulated FOXP3 reporter constructs, as demonstrated by site-specific mutations. We demonstrated that GATA-3 binds the FOXP3 promoter target

sequence not only in artificial DNA-protein binding assays, but also on the chromatin level in Th2 cells. Furthermore IL-4 inhibits FOXP3 mRNA expression in differentiating T_{regs}. Transgenic mice constitutively expressing GATA-3 in T cells are characterized by decreased FOXP3 expression and a lower frequency of $CD25^{+}FOXP3^{+}$ T_{regs}.

It appears that T_{regs} require antigen-specific stimulation as Th1 or Th2 cells but differentiate only in the absence of Th1 and Th2 driving signals. T_{reg} differentiation pathways may therefore be considered as a default pathway occurring in the absence of Th1/Th2 polarizing danger signals. Thus, therapeutic establishment of allergen tolerance requires the control of inflammation to facilitate regulatory capacities of the immune system in allergic disease.

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Group V Phospholipase A2 and Innate Immunity

Eriya Kikawada, Barbara Balestrieri, Jonathan Arm

While the essential function of the 85 kDa cytosolic phospholipase A_2 (cPLA-$_2$) alpha in providing arachidonic acid for eicosanoid generation is well established, the roles of the secretory phospholipase A_2 ($sPLA_2$) enzymes are poorly understood. To address the role of group V $sPLA_2$, which is expressed in mast cells and macrophages, we generated mice in which the gene encoding this enzyme was disrupted by homologous recombination. We also used an antibody specific to group V $sPLA_2$ to study its subcellular location. Prior to zymosan stimulation, group V $sPLA_2$ co-localizes with markers of the Golgi apparatus and the recycling endosome in mouse peritoneal macrophages. Following ingestion of zymosan, group V $sPLA_2$ is recruited to the phagosome. Furthermore, $cPLA_2$ alpha, 5-lipoxygenase, 5 lipoxygenase activating protein, and LTC_4 synthase were each recruited to the phagosome after ingestion of zymosan where we demonstrated cysteinyl leukotriene formation. Eicosanoid generation in response to zymosan was attenuated ~50% in group V $sPLA_2$-null macrophages, and the early phase of plasma exudation in zymosan-induce peritonitis was markedly attenuated in group V $sPLA_2$-null mice. Furthermore, peritoneal macrophages from group V $sPLA_2$-null mice, but not from mice lacking LTC_4 synthase or $cPLA_2$ alpha, demonstrated a ~50% attenuation of phagocytosis of zymosan particles that was restored by adenoviral expression of group V $sPLA_2$ but not group IIA $sPLA_2$. In separate studies we found that mast cells derived from mice lacking group V $sPLA_2$ have ~50% attenuation of eicosanoid generation in response to TLR2 agonists, but not to IgE and antigen. Furthermore, group V $sPLA_2$-null mast cells have significant attenuation of the phosphorylation of p42/44 ERK and $cPLA_2$ alpha in response to TLR2 stimulation, providing a mechanism whereby group V $sPLA_2$ amplifies the essential function of $cPLA_2$ alpha in regulating eicosanoid generation in response to an innate immune stimulus. Thus, we provide novel data demonstrating that the phagosome is a site of leukotriene biosynthesis. We further show that group V $sPLA_2$ contributes to the innate immune response and inflammation through the regulation of phagocytosis and eicosanoid generation.

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Biphasic itch stimulus model for investigations using functional magnetic resonance tomography (fMRT)

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Itch is a crucial symptom of allergic skin disease with difficult objective measurement. fMRT studies on itch have been hampered by the lack of a phasic stimulus. We present a short-term temperature-modulated human histamine itch model and results from its use investigating the cerebral activation in fMRT.

In 9 healthy right-handed volunteers (age 29±2.6 y), 1% histamine dihydrochloride was used as evaluated itch stimulus on the right forearm with subsequent modulation of the target skin area temperature by a Medoc TSA II thermode in 12 cycles (32°C to 25°C). Subjective scales were recorded using a computerized visual analog scale (VAS) and the Eppendorf Itch Questionnaire (EIQ). This model served as stimulus paradigm in an fMRT study in 12 healthy volunteers, correlating subjective scales and significantly activated brain areas.

All subjects reported localized itch without pain; mean VAS itch intensity was 51±3.5% during cold and 34±4% during the relative warmth phases ($p<0.0001$). Also, mean EIQ ratings were significantly higher regarding the cold phases of every cycle. The fMRT study, comparing temperature-modulated stimulus phases with and without histamine, showed itch-related activation in thalamus, anterior insular, inferior parietal and lateral prefrontal cortex (SPM2, RFX, paired t-test, $p<0.001$). VAS itch intensity was positively correlated to the posterior cingulate cortex and negatively correlated to the amygdala (SPM2, $p<0.001$).

In spite of the common knowledge that intensive cold inhibits itch sensation, a reproducible, significant enhancement of histamine itch by short term cooling could be shown. This effect might be explained by peripheral and central adaptation processes triggered by abnormal afferent activity patterns and was successfully used as phasic fMRT stimulus paradigm. Itch is processed by different brain structures contributing to the encoding of multidimensional aspects of itch. The subjective experience of itch is highly correlated with limbic structures known to be involved in emotional processing of unpleasantness and aversion.

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Novel genes distinguishing inhalant allergen-specific T cell memory responses in atopics

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Rationale: Atopic asthma is associated with hyperexpression of Th2 cytokines by aeroallergen-specific T memory cells. However, trials with recently developed Th2 inhibitors have proven disappointing, suggesting underlying complexities in atopy pathogenesis which are not satisfactorily explained via the classical Th1/Th2 paradigm. One likely possibility is that additional Th2-associated genes which are central to disease pathogenesis remain unidentified.

Objective: To identify novel Th2-associated genes associated with atopy.

Results: In contrast to published microarray studies in atopy which have focused primarily on mitogen activated T cell lines, we have concentrated on PBMC derived primary T cells stimulated under more physiological conditions of low dose aeroallergen exposure. We screened initially for gene expression by Affymetrix microarray, and validated genes of interest by quantitative RT PCR. We demonstrate an early wave of novel signalling-associated genes in atopic T-memory responses which cluster with IL4 and IL4R, followed by a later wave comprising unknown and known genes including those encoding classical Th2 effector cytokines IL5, IL9 and IL13. We further demonstrate that the same panel of novel Th2 genes also upregulate in response to another atopy-associated physiological stimulus superantigen SEB, but they remain quiescent in non-physiological responses driven by potent mitogens. We additionally demonstrate major overlap between atopic and non atopic responses with respect to expression of Th1-associated genes. Direct comparison between the responses of non atopics to HDM and the classical Th1 responses of Mantoux-positive subjects to PPD revealed selective attenuation within the non atopic HDM response of genes encoding the potent cytotoxic genes IFNg and CCL8. The responses of non atopics to HDM may thus be more correctly classified as "modified" Th1, by analogy with the "modified" non pathogenic Th2 response to cat.

Conclusions: We have identified a range of novel genes involved in the initial stages of aeroallergen induced activation of Th2 memory cells in atopics. They represent logical candidates for more detailed immunological and genetic studies related to atopy development and asthma pathogenesis.

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CD8 T cells stimulate dendritic cells to secrete IL-18 that induces Th1 and inhibits Th2 cell differentiation in vitro and suppresses IgE responses in vivo

Mike Kemeny, Maria Salagianni, Matthew Thomas, Alistair Noble

One of the first functions ascribed to CD8 T cells was their capacity to suppress certain types of immune response, most notably IgE antibody responses. Investigation of this process has subsequently shown that one route through which CD8 T cells inhibit IgE is by stimulating IL-12 from DC that induces Th1 and inhibits Th2 cells. In this study we have determined the contribution of IL-18, a cytokine that synergizes with IL-12 to promote Th1 cell differentiation. Our results show that CD8 T cells from OVA-specific T cell receptor transgenic (OT-I) mice induced IL-18 (increased from 30 to 100 pg/ml) when cultured with $OVA^{257-264}$-pulsed dendritic cells (DC). *In vitro,* IL-18 synergized with IL-12 to promote differentiation of Th1 CD4 T cells which increased from 4 to 40% and Tc1 CD8 T cells that rose from 58 to 82%. Secretion of IFN-γ by CD4 T cells in these cultures increased from 10 to 140 ng/ml but from CD8 T cells only rose from 260 to 300 ng/ml following culture with IL-12 and IL-18. When cultured under Th2 promoting conditions, IL-12 and IL-18 reduced the proportion of Th2 CD4 T cells from 18 to 2% and IL-4 in the supernatant fell from 18 to 4 ng/ml. *In vivo*, OVA-specific CD8 T cells inhibited IgE responses (up to 98% suppression)

when transferred to wild type, but not IL-18$^{-/-}$ mice. CD8 T cell IgE suppression was restored in IL-18$^{-/-}$ mice by the transfer of 1 x 10^3-1 x 10^5 wild-type DCs. The importance of IL-18 as a regulator of IgE was confirmed using IL-18$^{-/-}$ mice immunized with OVA-alum that produced an earlier and more sustained IgE response than either wild type, IFN-$\gamma^{-/-}$ or IL-12$^{-/-}$ mice. Thus CD8 T cells appear to induce DC IL-18 that synergizes with DC IL-12 to promote differentiation of Th1 over Th2 cells and thus inhibit IgE responses.

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Characterization of the structure and proteolytic activity of the major house dust mite allergen

Background: Inhalation allergy to house dust mite is among the most prevalent allergic diseases worldwide. The clinically most important mite allergens are group 1 and 2. The group 1 allergens are intestinal cysteine proteases expressed with large pro-peptides that must be enzymatically removed to obtain the mature active proteins observed in mite extracts. However, a mixed cysteine-serine protease activity of nDer p 1 has previously been proposed. The three-dimensional structure and the substrate specificity provide crucial tools for understanding the allergenicity and biological activity of this allergen.

Methods: The structure of a recombinant *Dermatophagoides pteronyssinus* group 1 isoallergen in its pro form (rproDer p 1) was solved by X-ray crystallography. Enzyme kinetics were used to determine a substrate that is not hydrolysed by the serine proteases present in the mite extract. nDer p 1 was purified from mite extract to apparent electrophoretic homogeneity (fraction 1), which was further separated by soybean trypsin inhibitor affinity chromatography into two fractions, containing bound serine protease and unbound nDer p 1, respectively. Proteolytic activities in the three fractions were assessed and proteins were identified by mass spectroscopy.

Results: The mature region of proDer p 1 adopts a conformation similar to the mature form of other cysteine proteases (e.g. papain) suggesting that no major structural changes are induced by maturation. The pro-region adopts a unique fold that interacts with the active-site cleft and a large flanking area on the mature region. Der p 1 cleaves the substrate Z-Leu-Leu-Glu-MCA that seems to be specific for Der p 1 in the extract, which is in accordance with the substrate binding cleft observed in the structure. Finally, the serine protease activity in fraction 1 was unambiguously identified as originating from trace amounts of nDer p 3.

Conclusions: We show here the crystal structure of Der p 1 and the identification of a potential substrate sequence. This study furthermore demonstrates that Der p 1 possesses only cysteine protease activity and that the observed serine protease activity originates from contaminating nDer p 3.

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Plasticity of Histamine H_1 Receptor During Human Macrophage Differentiation

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Histamine plays a pivotal role in allergic inflammation by inducing acute effects such as vasodilatation, increased vascular permeability and bronchoconstriction. In addition, histamine modulates cytokine/chemokine production and antigen-presenting function in monocytes, macrophages, T cells and dendritic cells. However, the responses elicited by histamine are extremely variable, suggesting that the expression of histamine receptors (H_1, H_2, H_3 and H_4) may be different between cells of the monocyte/macrophage lineage. We have examined the expression of the H_1 receptor in human peripheral blood monocytes and macrophages purified from the lung tissue or differentiated *in vitro*. mRNA expression for H_1 receptor was significantly higher in the lung macrophages and monocyte-derived dendritic cells than it was in monocytes. This observation raised the hypothesis that differentiation of monocytes into macrophages may induce upregulation of H_1 receptor. We therefore examined the expression of the H_1 receptor in a model of macrophages developed *in vitro* by incubation with 20% fetal calf serum (FCS) for 7 to 10 days. Quantitative RT-PCR experiments showed that the mRNA for the H_1 receptor increased 15-fold in differentiated macrophages as compared to blood monocytes. Western blot analysis indicated that the H_1 receptor protein expression increased more that 10-fold in macrophages. Histamine induced a concentration-dependent production of IL-8 in macrophages, whereas this response was barely detectable in the precursor monocytes. This effect was mediated by the H_1 receptor since it was completely abolished by preincubation of macrophages with levocetirizine, but not with ranitidine. The enhanced production of IL-8 in macrophages was not due to an increased capacity of these cells to produce cytokines since the release of IL-8 induced by lipopolysaccharide was comparable in monocytes and macrophages. These data indicate that differentiation of monocytes into macrophages, either *in vivo* in the lung or *in vitro,* is associated with upregulation of the histamine H_1 receptor. These observations suggest that histamine H_1 receptor expression can be modulated on cells of the monocyte/macrophage lineage. The expression of H_1 receptors on these cells profoundly influences their functional responses to histamine.

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